



ABSTRACT BOOK

SETAC EUROPE 32ND ANNUAL MEETING

15-19 MAY 2022 | COPENHAGEN, DENMARK
"TOWARDS A REDUCED POLLUTION SOCIETY"



Abstract Book

SETAC Europe 32nd Annual Meeting

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This book compiles the abstracts from the 32nd annual meeting of the Society of Environmental Toxicology and Chemistry – Europe (SETAC Europe), conducted from 15–19 May 2022 in Copenhagen, Denmark, and online.

The abstracts are reproduced as submitted by the author and accepted by the scientific Committee. They appear in order of abstract code and alphabetical order per presentation type. The poster spotlight abstracts are included in the list of poster abstracts. The presenting author of each abstract is highlighted in bold.

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Society of Environmental Toxicology and Chemistry Europe (SETAC Europe)

About SETAC

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, managers, engineers or others interested in environmental issues. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void and quickly saw dynamic growth in the Society's membership, meeting attendance and publications.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers of the World Council and Geographic Unit Boards of Directors and Councils, and in the composition of committees and other society activities. The proportion of members from each of the three sectors has remained nearly equal over the years.

The Society is concerned about global environmental issues. Its members are committed to Environmental Quality Through Science®, timely and effective communication of research, and interactions among professionals so that enhanced knowledge and increased personal exchanges occur. Therefore, SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.

SETAC's growth is reflected in the founding of Geographic Units around the world. SETAC Europe was established in 1989 as an independent organisation, followed by SETAC Asia-Pacific in 1997 and SETAC Latin America in 1999. In 2002, the four existing organisations joined together under the governance of the SETAC World Council. SETAC Africa is the most recent Geographic Unit, which was adopted in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters and branches have emerged in a number of countries.

SETAC publishes two journals, *Environmental Toxicology and Chemistry* (ET&C) and *Integrated Environmental Assessment and Management* (IEAM). ET&C is dedicated to furthering scientific knowledge and disseminating information on environmental toxicology and chemistry, including the application of these sciences to risk assessment. Integrated Environmental Assessment and Management focuses on the application of science in environmental decision-making, regulation and management, including aspects of policy and law, and the development of scientifically sound approaches to environmental problem solving. Together, these journals provide a forum for professionals in academia, business, government and other segments of society involved in the use, protection and management of the environment for the enhancement of ecological health and human welfare.

SETAC books provide timely in-depth reviews and critical appraisals on scientific subjects relevant to understanding a wide range of contemporary topics pertaining to the environment. These include any aspect of environmental chemistry, toxicology, risk assessment, risk management or environmental policy.

SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1992, and in Brussels, Belgium, established in 1993.

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Environmental Quality Through Science®

Track 1: Ecotoxicology and human toxicology: From molecules to organisms, from omics to in vivo

1.01 Advantages of using lab and field collected invertebrates in ecotoxicology: Challenges and opportunities for Environmental Risk Assessment (Part I)

1.01.T-01 Protein Binding on Acutely Toxic and Non-Toxic Polystyrene Nanoparticles During Filtration by *Daphnia magna*

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Nanomaterials can adsorb biomolecules to their surface and form a protein corona. In the present study, we investigated the protein profile bound to different sizes of aminated and carboxylated polystyrene (PS) nanoparticles after passing through the digestive tract of the freshwater zooplankton *Daphnia magna*. We found that acutely toxic aminated 53 nm PS nanoparticles binds a different set of proteins compared to other non-toxic PS nanoparticles. A higher amount of proteins appeared to be bound to the aminated PS nanoparticles. The fact that the aminated nanoparticles bound a larger number of proteins, agrees with the aggregate size results. The protein bound to toxic nanoparticles can be divided into two groups. One group of proteins which function is related to the digestive system, whereas the other group of proteins can be related to the epithelium, intracellular structures, and processes. Finally, we observed that not only proteins bind to surfaces of the nanoparticles. Triglycerides effectively bind to 200 nm carboxylated PS nanoparticles but not to the other tested nanoparticles. These results provide information about the composition of the corona formed on surfaces of nanoparticles after short-term (4 h) incubation with *D. magna*.

1.01.T-02 High-Throughput Screening of Phototactic Behaviour in *Daphnia magna*: From Vertical to Horizontal Responses to Light

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Animal behavioural trait responses are increasingly being used in environmental risk assessment. Nevertheless, behavioural responses are still hampered by a lack of ecological relevance and standardisation. This is the case for diel vertical migration of zooplanktonic species. Previously we developed a vertical oriented behavioural hardware able to reproduce phototactic fish induced depth selection in *Daphnia magna* and its modulation by fish kairomones and psychotropic drugs. This study aims to test if it is possible to use an horizontal 24 multi-well plate maize set up to assess phototactic fish induced responses in *D. magna*. The study was conducted using two clones with opposed phototaxis upon exposure to fish kairomones and using psychotropic drugs known to modulate phototaxis. Acrylic strips opaque to visible light but not to the infrared one were used to cover half of the arena of each of the wells of the multi-well plate. Clone P_{132,85} showed positive phototaxis in either the vertical and horizontal set up and negative phototaxis when exposed to fish kairomones or to the muscarinic acetylcholine receptor antagonists scopolamine and atropine. The opposite behavior was observed for clone F. Diazepam and pilocarpine ameliorate fish kairomone induced negative phototaxis and picrotoxin increased it only on clone P_{132,85} in the vertical set up. The study of neurotransmitter metabolites showed much greater concentrations of dopamine and of glycine in clone F, which may be relate to its negative phototaxis and its observed lower responsiveness to fish kairomones. The results from this study suggest a simple, fast, high throughput assay for *D. magna*, and gains insight on the impacts of natural and psychotropic compounds on the swimming behaviours of a model crustacean species used in ecotoxicology studies.

1.01.T-03 Differential Sensitivity and Synergy in Adult and Larvae Lepidoptera

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Differential sensitivity to pesticides between insects may have many biological explanations. One such explanation may be the potential of a species to metabolise the toxicant. Therefore, an awareness of the presence of metabolic enzymes such as cytochrome P450's (CYPS), glutathione-S-transferases (GSTs) and carboxylesterases in a specific species may hold an insight into the potential sensitivity or tolerances the species is likely to have towards chemical toxicants. Here we aim to determine the toxicity of the insecticide cypermethrin and the fungicides azoxystrobin, chlorothalonil and prochloraz to adult and larval stages of the cabbage moth (*Mamestra brassicae*). Using single chemical toxicity data and TKTD modelling, we aim to identify cases of synergism between cypermethrin and the fungicides in binary mixtures. Finally, we begin to explore any differences in sensitivity using the levels of expression of CYPS, GSTs and carboxylesterases in the transcriptome of the larvae and adult. Single chemical tests were analysed using the GUTS_RED_SD model and revealed fungicides had no effect on mortality on cabbage moth larvae. Therefore, no LC₅₀'s could be calculated for azoxystrobin, chlorothalonil or prochloraz. However, the insecticide cypermethrin had an LC₅₀ of 46.3 and 11.6 at 48 and 96 hours respectively. This differed in adult moths as there was a greater tolerance to cypermethrin with LC₅₀ values of 90.5 at 48 hours and 22.7 and 96 hours, a difference of almost 50%. Larval mixture testing identified slight synergy in larvae between cypermethrin and prochloraz and a more pronounced synergistic interaction between cypermethrin and chlorothalonil. No synergism was seen between azoxystrobin and cypermethrin. Adult mixture tests are on-going but it is hypothesised that synergistic effects will be lessened as is seen in the single chemical cypermethrin test. Further, a catalogue was created of CYPS, GST's and carboxylesterases from the transcriptome of cabbage moth larvae. The adult moth transcriptome is underway and will be compared against the larval transcriptome. We hypothesise that differences in sensitivity may begin to be explained by differences in expression of metabolic enzymes. We hope that this multi-faceted approach of lab exposure, GUTS modelling and transcriptomics provides an insight into the power of omics as a predictive tool of toxicity, particularly when used alongside traditional toxicity testing.

1.01.T-04 Experimenting the Plasticity of Sensitivity From Field-Populations of *Gammarus fossarum* in Response to Chronic Cadmium Contamination: Tolerance Acquisition, Transfer and Reversibility

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Chronic exposure to metal contamination can induce changes in the sensitivity of populations. An increase in tolerance, transmissible between generations, can be observed in certain cases without being due to genetic adaptation. This particular case of transgenerational acclimatization using non-genetic mechanisms of heredity has been documented in natural populations of the crustacean *Gammarus* historically exposed to metallic geochemical background. In these populations, the acquisition of cadmium (Cd) tolerance has been shown to be consistent with the manifestation of parental effects of exposure. The objectives of our study were then to confirm the existence of Cd tolerance among several *G. fossarum* populations at a regional scale, to better understand the nature of this tolerance acquisition (transitory acclimatation or permanent acquisition of tolerance by the organisms) and to study the mechanisms of transmission between generations. For that, natural Cd-contaminated and uncontaminated field *G. fossarum* populations was used in three laboratory protocols, 1- the returning in clean water in the lab of Cd-contaminated and uncontaminated field *G. fossarum* populations to question the reversible nature of sensitivity, 2- the re-matching spawners from Cd-tolerant and sensitive field populations to study the transfer of the tolerance to progeny and, 3- the establishment of a Cd chronic exposure in the lab at environmentally relevant concentrations to test the mechanisms seen in naturally tolerant populations. This study confirms the existence of natural populations capable of developing mechanisms of tolerance in Cd contaminated environments. Moreover, our results show that tolerance is not fixed for the life time of individuals, not depending therefore on genetic mechanisms or developmental plasticity, while this tolerance can be transmitted between generations by parental effects with a symmetrical role of the two sexes. Thus, the use of invertebrates is of real interest to increase our understanding of long-term effects of chemical contaminations in ecosystems by allowing to conduct environmentally relevant ecotoxicological studies.

1.01.T-05 Predicting the Survival of Field Gammarids Provides Additional Insights on the Impacts of Fluctuating Pesticide Exposures

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The analytical detection and modelling approaches to assess pesticide exposure in various environmental compartments have substantially improved over the last several decades. However, the threshold values considered in the current regulatory risk assessment are still based on laboratory studies that do not mirror the conditions captured in chemical monitoring and modelling. In this study, we employed a toxicokinetics-toxicodynamic (TKTD) model from the General Unified Threshold model of Survival (GUTS) framework to predict the impact of fluctuating pesticide concentrations on the survival of gammarids (*Gammarus pulex*) in an agricultural stream located in Switzerland. Individual exposure to azoxystrobin, chlorpyrifos and diazinon were predicted to have an acute and/or chronic impact on the survival of gammarids. In contrast, exposure to imidacloprid and propiconazole were predicted to have a low impact as their environmental concentrations must be multiplied by more than 100 before a survival probability of 50% is observed. Our modelling approach subsequently revealed that the duration of exposure drives the effects more than the maximum concentration as typically considered in traditional risk assessments. We further extended the GUTS model to incorporate mixture effects using concentration addition. We found that a considerable impact is expected when gammarids are exposed to organophosphates (chlorpyrifos, chlorpyrifos-methyl, and diazinon), whereas a mild impact is predicted for neonicotinoids (imidacloprid and thiacloprid). Given that the gammarids are exposed to many other environmental contaminants and stressors, a decline in their survival probabilities because of chemical stress will likely influence their individual fitness and/or overall population viability. Additional laboratory and/or field studies are required to validate the mixture assumptions in our model. However, by incorporating the temporal aspects of environmental exposure and the associated consequences on survival, our study added a layer of realism that improves the current risk assessment of pesticides.

1.01 Advantages of using lab and field collected invertebrates in ecotoxicology: Challenges and opportunities for Environmental Risk Assessment (Part II)

1.01.T-06 Biomarker Development in High-Resolution Pesticide Mixture Exposures Using the Springtail *Folsomia candida*

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Invertebrate populations worldwide are in decline, at least partly due to intensive pesticide application. Traditionally, pesticide risk assessment has been based on the results of standardized tests measuring the effects of a single pesticide on the reproduction or survival of model organisms. It is difficult to extrapolate these findings to field conditions as most agricultural soils are polluted by pesticide mixtures and the effects of their (synergistic) interactions on invertebrates are largely unknown. Gene regulatory patterns could be used as biomarkers to determine the type of soil pollution present and its intensity under varying mixture compositions. Generating high-throughput gene expression data of high-resolution exposure intensities, and the development of methods to analyse this data are crucial for linking gene-expression patterns to adverse effects on reproduction, and for subsequent implementation of biomarkers in risk assessment or biomonitoring. In this study, transcriptomic data was obtained for the soil ecotoxicological model species *Folsomia candida* (springtails) under a high-resolution grid-design-exposure of either two

neonicotinoids, imidacloprid and clothianidin, or imidacloprid and cyproconazole, a fungicide. In total the exposure consisted of 168 samples over 25 unique conditions in two experiments. Obtained gene expression data on all pesticides was analysed simultaneously with a joint Gaussian processes (GP) additive model, as GP-models allow for the analysis of non-linear differential gene expression patterns in a grid design. In total, this method identified 2049 differentially expressed genes (DEGs) under the exposure of neonicotinoids and 1080 DEGs under the exposure of cyproconazole. Gene set enrichment (GSE) analysis reveals shared and distinctive gene functions for DEGs under neonicotinoid and cyproconazole exposure, which can be used to identify points of synergistic interaction or used for biomarker development respectively. The candidate biomarkers are identified from distinctive GSE terms and validated on agricultural soils, providing risk assessors with metrics of adverse effects of pesticide presence that can be used on field-relevant mixture exposures.

1.01.T-07 Functional Transcriptomic Fingerprints of Neurotoxic Modes-Of-Action in *Daphnia magna*

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Active ingredients of pesticides, biocides or pharmaceuticals can cause undesirable side effects in the aquatic ecosystem. Therefore, an environmental hazard and risk assessment is required prior to the registration of new active substances. The freshwater crayfish *Daphnia magna* is a model organism for evaluating acute and chronic toxicity in aquatic invertebrates. However, standardized tests using *Daphnia* are limited to immobility and reproductive endpoints and thus provide limited insight into underlying modes-of-action (MoA). Here, we applied transcriptome profiling to a modified acute immobilization assay of *D. magna* to analyze and compare gene expression profiles induced by the GABA-gated chloride channel blocker fipronil and the nicotinic acetylcholine receptor (nAChR) agonist imidacloprid. To detect compound-induced gene expression changes at low effect concentrations, an acute immobilization test with *D. magna* was performed following the corresponding OECD guideline test. 48 hours after test initiation, total RNA was extracted from the daphnids and purified polyA-RNA was subjected to RNA sequencing for differential gene expression analysis. The observed signatures for fipronil and imidacloprid were readily distinguishable between the two compounds at the gene level without major overlap. While fipronil exposure affected a number of metabolic pathways, including ATP synthesis and cholesterol biosynthesis, metabotropic glutamate receptor signaling, and a pathway associated with Alzheimer's disease - the presenilin pathway in mammals, impaired imidacloprid pathways associated with Parkinson's disease, mammalian vasopressin synthesis, GABA synthesis, and aminobutyrate degradation. Our study suggests that the integration of transcriptomics into a modified version of the acute immobilization assay of *D. magna* can be used to differentiate modes-of-action of test compounds of interest at low effect concentrations. Such a combination of systems biology methods with ecotoxicological test guidelines will help to understand the underlying molecular mechanisms of adverse effects on invertebrate organisms and populations, which will be important for ecotoxicological hazard assessment.

1.01.T-08 Physiological Colour Changes in Ecotoxicology

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Many animals display rapid colour changes for the purpose of camouflage. Physiological colour changes can be controlled either nervously, as in cephalopods, or hormonally, as in crustaceans. Both, neuronal and neurohormonal control mechanisms are regulated by neurotransmitters. In cephalopods, serotonin induces the relaxation of the muscle crown leading to pigment concentration in the chromatophores. In crustaceans, dopamine stimulates pigment concentration *via* red pigment concentrating hormone, whereas serotonin ensures pigment dispersion *via* pigment dispersing hormone. Environmental pollutants can potentially interfere with these mechanisms of physiological colour change and cryptic behaviour. We exposed *Carcinus maenas*, *Crangon crangon* and *Sepia officinalis* to waterborne selective serotonin and/or norepinephrine reuptake inhibitors, which are known to alter the levels of serotonin and other biogenic amines in wildlife. Venlafaxine provoked pigment concentration in chromatophores of *S. officinalis* at the same concentration (10^{-4} M) as did serotonin. Fluoxetine alone (5 ng L^{-1}) or in combination with venlafaxine (2.5 or 5 ng L^{-1}) improved uniform, but not disruptive body patterns in newly hatched cuttlefish. *C. crangon* exposed to 10 - 1000 ng L^{-1} fluoxetine for up to one week showed a significant increase in darkening following 30 min on black substrates. Similarly, pigment dispersion in juvenile *C. maenas* transferred from a white to a dark background tended to increase when exposed to fluoxetine at 5 ng L^{-1} , but when exposed to a combination of fluoxetine and venlafaxine at 5 ng L^{-1} each, darkening was significantly impaired. These studies demonstrate that physiological colour changes are susceptible to environmental chemicals that interfere with the neuronal or neurohormonal control of chromatophore movements. Hence, they may serve as indicators of environmental pollution and could point to detrimental effects on behavioural ecology that are important for the animals' survival. Colour change bioassays, therefore, provide a promising tool for environmental testing with the advantage of being non-invasive, inexpensive and relatively straightforward.

1.01.T-09 Organophosphate Pesticides in Aquatic Ecosystems: Study CASE on *Chironomus riparius* Impact at MOLECULAR Level

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Organophosphate pesticides (OPPs) have gained worldwide concern by their persistent and hazard to the environment, surrounding nearly 40% of the global market and in constant increase reaching easily to aquatic systems acting as main OPPs reservoir. Chlorpyrifos (CPF) insecticide has been the most employed for crops protection in Italy derived in intensify presence in surface waters (0.12-15.38 ng/L) along the country. The harmful CPF impact is widely documented reaching to be banned by EPA in August 2021, although as a semi-persistent pollutant, continue to be present in aquatic systems for years. However, the impact on field organisms has been poorly studied apart from our previous experience on *Diamesa* species. Therefore, this work is focused to evaluate the CPF impact at molecular level on *C. riparius* larvae collected in Rio Gola stream (32T 664117 5100540 UTM, October 2020), to check the influence on benthic macroinvertebrates in close contact with polluted sediments. The larvae were exposed at 0.011 µg/L, 0.11 µg/L and 1.1 µg/L for 24 and 72 h, in controlled laboratory conditions (8 light:16 dark hours; 20 °C). Sub-organismal response was evaluated using an array (48 genes) designed for this specie by Real Time PCR. After 24h, *Dis*, and *NHR38* (endocrine system); *GSTo1* (detoxification response); *Proph* (immune system); *XRCCI* (DNA repair), and *LIP* (lipid metabolism) were altered. At 72h higher response was observed *JHAMT*, *Dis*, *NHR38*, *NHR96*, and *FKBP39* (endocrine system); *Cyp4c1*, *Cyp12a1*, *GSTd6* (detoxification response); and *hsp70* (stress response) modulated. Hormone's synthesis inhibition confirmed the endocrine disruptor CPF capacity. Moreover, phase II modulation reinforces the role of CPF as oxidative compound, being confirmed by the immune system activation strongly related to ROS. Lipid metabolism was modulated could limit insect's functions because are essential source of energy (i.e: reproduction). Besides, the DNA damage seems to be drastic at 72h with apoptosis initiation (*Decay*). Finally, the stress response could indicate defective protein folding. Our findings confirm the risk of CPF, at environmental relevant concentrations, affecting the metabolism and future survival on a key aquatic organism. The differences in expression observed between 24 and 72h reinforce the importance to check the molecular modulation along the time in above all organisms as *C. riparius* with short life cycles.

1.01.T-10 How Nano Pesticides Affect Non-Target Organisms: Toxicity of Three Nanopesticides on *Daphnia magna*

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Nanopesticides as a potential solution to solve the risk and drawbacks of conventional formulations of pesticides have been attracting attention in both research and market areas. However, the risk of these new formulations still needs to be studied on non-target species and regulated specifically. In this regard, we prepared three nanopesticides composed of nanoparticles of polycaprolactone (PCL), polyhydroxybutyrate (PHB) and nanostructured lipid carrier (NLC) loaded with the fungicide tebuconazole (TBZ (a.i.)), as well as three suspensions of the nanocarriers without a.i. Then, we assessed the toxicity of all the nanoformulations to an aquatic model, *Daphnia magna*, through immobilization bioassay. For that, in total 28 neonates < 24h old in four replicates were exposed to 5 dilutions of each nanopesticides obtaining a total TBZ concentration of 36, 18, 9, 4.5 and 2.25 µg mL⁻¹. Likewise, the toxicity of the nanocarriers without a.i. was tested as control. The aim is to understand how *Daphnia magna* behaves in different concentrations of a.i. associated with nanocarriers and in the presence of the nanocarriers without a.i. over 48h. The total concentrations, release rate of a.i. and the stability of NPs at time 0 and 48h were checked. According to the results, *D. magna* reacted differently to exposure with each NPs. The dose-response results showed that the EC50 values of all the nanopesticides were high compared to the value for pure TBZ, which shows that the toxicity of all nanopesticides was lower than that of pure TBZ. The toxicity of the NPs decreased depends on the type of nanocarrier as follows: PCL-TBZ < PHB-TBZ < NLC-TBZ. The toxicity of the nanocarrier without a.i. was diverse. As, PCL showed some toxicity while PHB nanocarrier had almost no effect on the immobilization of *D. magna*. Consequently, since the toxicity of the tested NPs were less than that of pure TBZ, they can be a promising solution to address the concern regarding the risk of TBZ in one hand and in other hand as they showed improvement of a.i. solubility. These results are the effect of a 48h exposure of *D. magna* to nanopesticides. Therefore, longer-term exposure and reproduction bioassays are important for robust results, which they are ongoing. In general, as the different nanopesticides and their nanocarriers in the present study showed different responses, it highlights the importance of relevant guidelines for environment risk assessment of nanopesticides specifically.

1.01 Advantages of using lab and field collected invertebrates in ecotoxicology: Challenges and opportunities for Environmental Risk Assessment (Poster)

1.01.P-Mo001 The Comet Assay With Standard Aquatic Test Organisms As an Alternative Test System for an Environmental Risk Assessment of Pharmaceuticals

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Human pharmaceuticals find their way into aquatic ecosystems e.g. via municipal wastewater, where they pose a potential threat to aquatic organisms. A comprehensive environmental risk assessment (ERA) is necessary to minimize their risks. In accordance to the guidance on human pharmaceuticals for an ERA, effects on aquatic organisms are determined based on standardized guidelines including, among others, the *Daphnia magna* Reproduction Test (OECD 211). The EMA guideline for ERA was published in 2006 based on the efficacy of active substances in previous years. Meanwhile there are more potent active

substances, putting into question whether the test systems still offer sufficient protection for aquatic organisms. In a previous project (German Environmental Agency, FKZ 3718 65 420 1) alternative test systems for an ERA were identified. One of the identified test systems was the Comet Assay, a genotoxicity test that quantifies DNA damage by measuring the fraction of DNA that migrates out of a nucleus during gel electrophoresis (Tail intensity or TI%), with environmentally relevant organisms. Therefore, we established the Comet assay with the invertebrate *D. magna* and tested the effect of several pharmaceuticals either used in oncology (e.g. Cyclophosphamid) or cardiologica (e.g. Edoxaban) to account for genotoxicity as a potential adverse effect of pharmaceuticals. *D. magna* were exposed for 48 hours in accordance to the OECD 202 with test concentrations chosen to result in sublethal effects. After exposure, immobility was recorded and immobile daphnids were discarded. *Daphnia* cells were isolated and purified via filtration. The cells were mixed with agarose and pipetted onto microscopy slides. The slides were then incubated in a lysis solution to remove membranes. A treatment in an alkaline solution was performed causing the DNA to unwind and separate into single strands. Gel electrophoresis was performed and the slides were washed and dried overnight. The gels were stained using the DNA dye Vista Green and examined under a fluorescence microscope. Images were evaluated using the software CaspLab in order to determine tail intensity (TI%) values. The comet assay on *D. magna* was successfully established. In a calibration with hydrogen peroxide an increase in median TI% of over 60% was determined confirming that the method is capable of detecting genotoxicity. Of the pharmaceuticals tested so far, only Edoxaban showed a dose dependent increase in TI%.

1.01.P-Mo002 Impact of Carbon Nanotubes on an Emerging Cellular Organism: Physarum polycephalum

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Carbon nanotubes (CNTs) are a category of nanomaterials with applications in all fields (energy, transports, composite materials and environment) due to their exceptional mechanical, optical and electronic properties. However, their use should depend on the non-toxicity of these nanomaterials, and little is still known on their impact on living beings^{1,3,4}. This study evaluated the toxicity of double-walled carbon nanotubes (as a model of CNTs in general) on the unicellular organism *Physarum polycephalum*, which, due to its simplicity of organization and behavioural complexity², is a model of choice. The objective was to assess the fate of CNTs within the cell of *Physarum polycephalum*, and to evaluate their impact on the behaviour of the latter. For this purpose, organisms were contaminated along three routes of exposure (topical, food, environment) in order to determine the internalization of CNTs. The food exposure was the more efficient. Accumulation and excretion of CNTs within the cell were then assessed only by food exposure of the organism. No bioaccumulation and a fast excretion from the cell were observed. From a behavioural point of view, impacts on the migration speed, the fusion process and the cognitive performance were also investigated.

1.01.P-Mo003 Polyethylene Impacts in Fatty Acids and Carbohydrate Profiles of the Estuarine Species *Scrobicularia plana*

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Microplastic (MP) pollution in marine environments is a topic of emerging concern. Due to their ubiquity and small size, these particles are easily internalized by marine organisms, causing adverse biological responses. Bivalves are considered bioindicators of environmental health due to their wide distribution, ability to filter large volumes of water, sessile behaviour and ecological and commercial importance. Biomarkers, such as fatty acids (FAs) and carbohydrates (CHs), have been recently proven to be powerful tools to detect and evaluate an organism response to contaminants' exposure. The present study assesses the biochemical (FAs and CHs content) and ecotoxicological responses of two size classes (big and small) of the estuarine bivalve *Scrobicularia plana* exposed to cospheric polyethylene (PE) microparticles (75-90 µm). Organisms were exposed to environmental relevant and also higher concentrations of virgin PE microparticles (30 particles L⁻¹, 90 particles L⁻¹, 150 particles L⁻¹, 450 particles L⁻¹) for 96h. Results showed that PE ingestion occurred at all concentrations and microparticles accumulated in the visceral mass of *S. plana*, with the small size being the most affected class. PE exposure caused significant decreases in polyunsaturated fatty acids (PUFAs) content, including some essential fatty acids (EFAs), which are important compounds for their growth, reproductive ability and overall fitness. Concerning CHs results, PE exposure significantly decreased xylose, galactose and glucose contents. The decrease in glucose content can be interpreted as an exploitation of glycogen reserves for glucose availability caused by the condition of stress induced by the presence of the pollutant. The decrease in long-chain fatty acids, such as PUFAs, might be a consequence of depletion of glycogen reserves. As available glucose declines, *S. plana* started exploiting other energy sources to compensate such loss of energy, triggering lipids hydrolysis. These alterations may affect the overall fitness of the organisms and may cause changes in their nutritive value and thus along the trophic food chain, with potential implications for human health. As bivalves are consumed without removal of the digestive tract, they represent an important route of MP exposure for humans and ecotoxicological data on the effects of MPs are urgently needed as potential consequences on human health are still unknown.

1.01.P-Mo004 Polyethylene Impacts on the Antioxidant Defence System of the Estuarine Species *Scrobicularia plana*

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Plastics have become a substantial part of modern life and ubiquitous in all environments. Microplastics (MPs) are emerging

contaminants in marine environments and pose a serious threat to the marine ecology. MPs arise from land sources, deterioration of larger plastics, or intentionally manufactured as “primary” or “virgin” microbeads. MPs can be found in diverse shapes and sizes, and their long-term effect is yet unknown. Estuarine ecosystems, as transitional systems between marine, fluvial, and terrestrial environments, represent a hotspot for MPs pollution, where sediments are most likely the final sink. MPs ingestion has been reported in a wide variety of marine species, still the ecotoxicological effect on marine ecosystems is poorly understood. The benthic bivalve *Scrobicularia plana* was selected for its capacity to filter pollutants and the role it plays in the functioning of estuarine communities. For this study, organisms of two size classes (big and small) were exposed to relevant environmental virgin polyethylene (PE) microparticles (75-90 μm) and also higher concentrations (C1 = 30 MPs L⁻¹, C2 = 90 MPs L⁻¹, C3 = 150 MPs L⁻¹, and C4 = 450 MPs L⁻¹) to assess the ecotoxicological and biochemical effects of this pollutant. A battery of biomarkers was selected to determine the ecotoxicological effects of PE microparticles and to assess the potential use of enzymatic biomarkers for PE bioassays, including the activity of antioxidant enzymes (glutathione peroxidase (tGPx), glutathione reductase (GRed), glutathione-S-transferase (GST), Catalase (CAT) and Superoxide dismutase (SOD)) and the lipid peroxidation occurrence (TBARS). PE microparticles did not show lethal effects for *S. plana*. However, alterations occurred in the activities of the antioxidant enzymes and lipid peroxidation. Clams of both size classes showed dose-dependent responses at the highest concentrations (450 MPs L⁻¹), significantly increasing the activities of GST and GRed. A trend is observed between the antioxidant enzymes CAT, SOD, and tGPx for both size classes. The results indicate the suitability of *S. plana* as an indicator for the presence of virgin PE microparticles in aquatic environments. Furthermore, this work reports that GST activity increases significantly under the highest concentration of MPs (450 MPs L⁻¹), suggesting to be the best enzymatic biomarker to assess the presence of PE microparticles.

1.01.P-Mo005 Toxicity of Cigarette Butts and Their Associated Chemicals on the Freshwater Invertebrate *Chironomus riparius*

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Cigarette butts are one of the most commonly found litter items in the global environment. Their distribution spans from streets to beaches, rivers and oceans. Once ignited, the smoke produced by the tobacco mixture contains many harmful substances such as nicotine, PAHs, formaldehyde, hydrogen cyanide, lead and arsenic. These substances are partially trapped into the cigarette filter, which, once present in the environment, can release the chemicals into the environment. While the effects of smoking cigarettes are well understood, the effect of cigarette butts on the environment is still to be better researched. The aim of this study was to investigate the effects of cigarette butt leachates on the aquatic environment. For this purpose, we used the model organism *Chironomus riparius* (midge larvae), a widely spread and abundant organism in freshwater ecosystems. The invertebrate larvae are in direct interaction with both sediment and water, making them representative of both compartments, an interesting trait for the experiments we carried out. Cigarette butts were left to leach in containers containing sand and freshwater. The two matrices were then separated. Chironomid larvae were exposed either to contaminated sand or contaminated water, complemented with clean water or sand, respectively. This experimental design allowed us to study where the cigarette leachates are more prone to accumulate and therefore which organisms, benthic or pelagic, would be most at risk. We also followed a multi-endpoint approach, exposing the organisms at different life stages and for different amounts of time (from 7 days to 3 months), assessing endpoints such as growth, development and teratogenicity. Our results show strong evidence that leachates from cigarette butts impact *C. riparius*. More pronounced effects were observed when comparing the sediment phase to the water phase. This indicates that chemicals contained in cigarette butts can leach and enter both the sediment and water phases, but with a stronger affinity to sediment, potentially putting benthic organisms more at risk. Benthic organisms are essential for ecosystem functioning. In cigarette polluted basins, a loss of benthic species might mean the collapse of an entire ecosystem. We suggest that more experiments be conducted on different species of benthic organisms in order to confirm that cigarette butts are particularly toxic to sediment dwelling organisms.

1.01.P-Mo006 Relevance of Mysid Shrimps Toxicity Tests for Risk Assessment in Europe

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Mysid shrimp (*Americamysis bahia*) shows high sensitivity to pollutants. This finding increases the impact of marine test systems on risk assessment. But it is not clear if this is also relevant for the European zone and if sensitivity of the mysid shrimps is comparable to common species in Europe. We present data of the sensitivity of the mysid shrimp *Americamysis bahia*, native to the east coast of the United States in comparison to species in the East and North Sea (e.g., *Praunus flexuosus*). These data might be useful to evaluate the relevance of mysid shrimps tests for the risk assessment in Europe.

1.01.P-Mo007 Biochemical, Physiological, and Reproductive Effects of Glyphosate (FAENA®) and 2,4-D (HERBIDEX®) on the Tropical Cladoceran *Ceriodaphnia rigaudi*

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Herbicides have contributed to increasing agricultural production, but the intensive usage of these chemicals can produce significant damage in the environment, mainly through the affection of no target species. The herbicides glyphosate and 2,4-D are used worldwide for weed control; the first is a non-selective contact herbicide that inhibits the synthesis of aromatic amino acids needed for protein formation, whereas 2,4-D is an auxin hormone that inhibits the cellular division of meristematic tissue, causing the reduction of apical growth in plants. Aquatic organisms are exposed to herbicides reaching the water by leaching, running-off,

or adsorption to particulate matter. *Daphnia magna* is a Cladoceran frequently used in toxicity studies. However, it is important to consider tropical species such as *Ceriodaphnia rigaudi*, a zooplankter distributed in México. The objective of this study was to determine the toxic effects that the mixture of glyphosate- and 2,4-D-based commercial formulations produce in the reproduction, physiology, and biochemistry of *C. rigaudi*. LC₅₀'s (48h) (25°C, 16:8h light: darkness) were determined to be 1.09 mg L⁻¹ for glyphosate and 233 mg L⁻¹ for 2,4-D. Then, a chronic toxic assay was carried out during a whole life cycle. The tested concentrations were 0.0625, 0.125, 0.25, and 0.5 Toxic Units (TU) of the mixture of FAENA® and HERBIDEX®. During the tests, samples of 30 neonates from each clutch were picked up to analyze effects. The concentration of proteins, carbohydrates, and lipids and the activity of the enzymes CAT, SOD, and GPx were determined for each clutch. The 0.5 UT mixture of herbicides significantly decreased longevity. There was a delay of one day at the age of first reproduction in 0.125 and 0.5 UT concentrations. The accumulated progeny and the number of clutches decreased significantly at 0.5 UT. Abortions increased as the concentration of the mixture did. The content of proteins increased in the highest concentrations (0.125, 0.25, and 0.5 UT). Carbohydrates decreased up to 80% compared with the control, and lipids were significantly different in the treatment of 0.125 UT. The activity of the SOD was stimulated twice, but the activity of CAT and GPx was inhibited. The simultaneous presence of two herbicides in the water may lead to increased toxicity, causing more negative effects on aquatic organisms than that expected in single exposures.

1.01.P-Mo008 Finding the Optimal Conditions for Ecotoxic Experiments With the Larvae of the Terrestrial Land Crab *Cardisoma guanhum*

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The land crab *Cardisoma guanhum*, one of the keystone species on the island of Puerto Rico, has a complex life cycle composed of a terrestrial adult stage and a larval aquatic stage; composed of zoea and megalopa stages. During this aquatic phase, *C. guanhum* zoeas are affected by environmental factors such as food availability, temperature, and salinity, affecting the metamorphosis during the larval stage. Given this, we established the goal of discovering the ideal conditions for the development of *C. guanhum* larval stages and determining the set of conditions necessary for developing phthalates bioassays. Gravid *C. guanhum* females were captured in the wild and transported to the laboratory. Zoeas were removed from the females and immediately transferred to the experimental vials. The zoeas were exposed to three different temperatures, three different salinities, and two different food regimes. The zoeas were also subjected to several treatments such as various water quality, buffers prepared with various water quality, and different glassware cleaning conditions. The survival was recorded in each of the experiments. We found that the zoea exposed to 35 ppt and temperatures between 21°C and 27°C showed the highest survival. The food regimes showed that an excess and constant supply of *Artemia salina* newly hatched nauplii showed a tendency to higher survival of zoeas. The exposure of *C. guanhum* zoeas to different water quality conditions, to buffers prepared with different water quality conditions, and to different glassware cleaning regimes showed no survival difference among the treatments. We concluded that we had identified the optimal conditions for future toxicity experiments with phthalate.

1.01.P-Mo009 Investigation of Different Food Sources for a Chronic Laboratory Test With *Cloeon dipterum*

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In routine toxicity testing or regulatory evaluation of chemicals, standard organisms that are easy to maintain are often used. These organisms are not always sensitive enough to ensure a correct evaluation of the chemical. Besides the standard organisms, there are non-standard organisms like mayflies which are very sensitive to certain chemicals. In recent years, there has been a growing interest in keeping a mayfly species in the laboratory for toxicity testing. An important aspect for keeping mayflies is the food source. It has been shown that the development rate of the mayfly larvae is directly related to the quality of the offered feed. To reproduce comparable results, the food source must provide enough energy for the larvae to develop well, and any mortality or developmental delay effect must be due to the substance being tested. In this work, different food sources for the mayfly species *Cloeon dipterum* were investigated regarding their practicability and quality. The tests were carried out with locally collected larvae of *C. dipterum*. For each test set 6 replicates with 5 larvae each were prepared. First two fish foods and periphyton cultivated on tiles were tested. Secondly, incubated poplar leaves as whole and as a powder were tested. Again, in parallel with periphyton. The experiments were stopped as soon as all larvae had emerged or had died. The development of the larvae was examined 3 times a week. In order to investigate an increase of mortality caused by the monitoring procedure, a further non-invasive test with periphyton tiles as food source was performed. Here, only the vitality was checked every 7 days. In the test with the artificial fish food 1 15% of the larvae emerged while, 85% of the larvae died. With the artificial fish food 2 only 55% mortality was observed. The parallel periphyton test showed the lowest mortality (30%). In the second run, the mortality in the periphyton test was higher (80%). Feeding with leaves resulted a slower development of the larvae, resulting in an increased duration of the test. The mortality was 55% - 60% and first dead larvae were observed early in the test. In general, larvae fed with fish feed developed slower compared to the test with periphyton tiles. Also, larvae died earlier and in smaller stages. In the non-invasive test, 40% of the larvae died. The regular examination of the larvae does not seem to have a negative influence on the test. Overall, the periphyton tiles turned out to be a promising feed for larvae of *C. dipterum*.

1.01.P-Mo010 Behavioural and Enzymic Activities Changes Under Chronic Exposure of Neurotoxic and Non-Neurotoxic Pharmaceutical in Benthic Invertebrate

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Among the emerging contaminants, pharmaceuticals are considered one of the most pertinent substances that may pose threats to

the aquatic ecosystems. Pharmaceuticals are designed to be directed at specific metabolic and molecular pathways in selected organisms. Thus, they are assumed to still be biologically active when entering the ecosystem and may therefore result in unpremeditated impacts on non-target organisms. Neurotoxic and non-neurotoxic pharmaceuticals have different mode of action and therefore cause different impacts on the organisms. In addition, environmental stressors, such as food availability and temperature, may interact with contaminant exposure to affect uptake and action of pharmaceuticals in organisms. An enhanced understanding of the mode of action of pharmaceuticals in aquatic organisms under different environmental stressors is crucial to assess the possible impacts at organism to population level. This project examines the chronic impacts of low concentrations of sediment-associated pharmaceuticals: Fenofibrate (lipid regulator), naproxen (nonsteroidal anti-inflammatory drug), fluoxetine and sertraline (antidepressants) to the deposit-feeding oligochaete, *Tubifex tubifex*, using non-conventional endpoints (i.e., burrowing, feeding activity) and enzymatic activity (e.g., AChE, GST) following chronic exposure under different environmental settings. The presentation will discuss the approach to construct Adverse Outcome Pathways (AOPs) for the selected pharmaceuticals: 1) a study on the changes of enzyme activities in *T. tubifex* under chronic exposure of neurotoxic and non-neurotoxic pharmaceuticals, 2) the use of non-conventional endpoints to assess the impact of chronic pharmaceuticals exposure on organisms, 3) an understanding of the impacts of environmental stressors on the mode of action of the pharmaceuticals and the response of the organisms, and 4) construction of AOPs to link behavioural and enzymic changes under chronic exposure of pharmaceuticals in benthic invertebrates.

1.01.P-Mo011 Effect of Diesel Particulate Matter on *Caenorhabditis elegans*: Neuro-Behaviour and Neurodegeneration *Nivedita Chatterjee*, INL International Iberian Nanotechnology Laboratory, Vila Nova de Gaia, Portugal

This study aims to elucidate whether the Diesel particulate matter (DPM) cause alterations in neuro-behaviours in *C.elegans*. Potential long-term toxicity (7 days) and acute toxicity (24h) were assessed in the dose range of 0.1 to 100 µg/ml. We found significant alterations in stress response, locomotion behaviour at sub-lethal dose. In connection with neuro-behavioural (dat-1) analysis, we found that marked impairment in dopaminergic function. *C.elegans* is an excellent model for neurodegenerative disease, such as Alzheimer's disease (AD), characterized by amyloid protein aggregation, otherwise soluble proteins. Our preliminary data evidenced that DPM cause amyloid protein aggregation in the model system (ongoing study). In summary, DPM exposure could impair neuro-behaviour and accelerate the ageing-related neurodegenerative disease in the model system.

1.01.P-Mo012 High Throughput Investigation of Amphipod Reproductive Behaviour

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Precopulatory pairing is a reproductive strategy in which a mature male holds a mature female until she moults and copulation occurs. The use of precopulatory pairing as a behavioural endpoint in ecotoxicology was first demonstrated in the 1970s where precopulatory pairing was used to determine mating success. This qualitative behavioural response can be measured as time of disruption of pairs, repairing time or post-dosing observation of pairs for reproductive success. Although this mechanism is sensitive, there has been a steady decline in its use in toxicity testing as it can be onerous. This study aims to provide an overview of past and current trends on the use of precopulatory pairing and discuss the optimisation of this technique using video tracking technology to evaluate the effects of plastic additives on the marine amphipod, *Echinogammarus marinus*. Pairs were separated and individuals were exposed to high concentrations (50 and 5000) µg/l of di-ethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP). The repairing activity was recorded using Zantiks MPV unit for 15 mins. The repairing time and the percentage of animals paired in the control and the exposed group were analysed. This approach to toxicity testing is a sensitive, reliable and cost-effective means of detecting the early warning effects of harmful environmental pollutants.

1.01.P-Mo013 Sertraline and Fluoxetine Chronic Exposure - Possible Sublethal Effects on the Key Benthic Invertebrate *Capitella teleta*

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Sertraline and fluoxetine are two selective serotonin reuptake inhibitors commonly prescribed to treat depression, anxiety, and post traumatic disorders in humans. Because Wastewater Treatment Plants (WWTPs) are not equipped to efficiently remove pharmaceuticals from wastewater, antidepressants are widely detected in the environment in the range of ng L⁻¹ and µg L⁻¹. These low concentrations might pose a risk to the resident species, especially in a long-term exposure. Since hydrophobic compounds easily sorb to organic matter, they tend to concentrate in sediments. Sertraline and fluoxetine are within these lipophilic chemicals, thus their presence in the water column could be less relevant than in sediment. However, not many studies have focused on sediment exposure of pharmaceuticals. Polychaetes are fundamental organisms for the geochemistry of benthic environments, and among them, *Capitella teleta* is a key species in organically enriched habitats, where it can reach densities of more than 100.000 individual/m². We tested the impact of sediment-associated fluoxetine and sertraline on conventional (e.g., mortality) and non-conventional (e.g., burrowing- and feeding behavior) endpoints. Since antidepressants are neurotoxic chemicals, we also assessed acetylcholinesterase (AChE) enzymatic activity changes. The results will be discussed.

1.01.P-Mo014 Integument Colour Change: Tracking the Inhibition of *Oppia Nitens* Growth As a Sub-Lethal Indicator of Soil Toxicity

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Growth is an important toxicity end-point in ecotoxicology but is rarely used in soil ecotoxicological studies. Here, we assessed

the growth change of *Oppia nitens* when exposed to reference and heavy metal toxicants. To assess mite growth, we developed an image analytic technique to measure colour spectrum change of the mite integument as a proxy for growth change. We linked the values of the red, green, blue, key-black and light colours of mites to different growth stages. Based on this concept, we assessed the growth change of mites exposed to cadmium, copper, zinc, lead, boric acid or phenanthrene at sublethal concentrations in LUFA 2.2 soil for 14 days. Sublethal effects were detected after 7 days of exposure. The growth of *O. nitens* was more sensitive than survival and reproduction when exposed to copper ($EC_{50_{\text{growth}}} = 1360 \text{ mg/kg}$ compared to $EC_{50_{\text{reproduction}}} = 2896 \text{ mg/kg}$). Mite growth sensitivity was within the same order of magnitude as mite reproduction, when exposed to zinc ($EC_{50_{\text{growth}}} = 1785$; $EC_{50_{\text{reproduction}}} = 1562 \text{ mg/kg}$). At least 25% sublethal effects of boric acid and phenanthrene were detected in the mites but growth was not impacted when *O. nitens* were exposed to lead. Consistent with previous studies, cadmium was the most toxic metal to *O. nitens*. The mite growth pattern was comparable to mite survival and reproduction from previous studies. Mite growth is a sensitive toxicity endpoint, ecologically relevant, fast, easy to detect, and can be assessed in a non-invasive fashion, thereby complementing existing *O. nitens* testing protocols.

1.01.P-Mo015 The Effects of Life Stage on the Sensitivity of *Folsomia candida* to Cyproconazole and Teflubenzuron

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To halt the loss of ecosystem services provided by terrestrial ecosystems, it is increasingly important to understand the effects of chemical stressors on the role of service providing units (SPU). Collembola are soil micro-arthropods with relevant roles in organic matter decomposition and nutrient cycling. For this reason, the registration of active ingredients used in plant protection products requires the assessment of their long-term effects on species of Collembola, such as *Folsomia candida*. The guidelines provided by the Organisation for Economic Cooperation and Development for such tests recommend the use of juvenile age-synchronized animals with 10 to 12 days old. In several studies these guidelines are modified by, e.g., using older animals at the beginning of the test, to compare the effects of an active ingredient with species of Collembola with slower life cycles. To determine the influence of the age of *F. candida* on the results of the toxicity test, we assessed the effect of two active ingredients on *F. candida* with an age difference of 10 days old. The fungicide cyproconazole (CYP) and the insecticide Teflubenzuron (TEF) were selected to clarify if the differences in sensitivity caused by the age of animals are observed for chemicals with distinct modes of action. After exposure to the chemicals, the dose-response curves obtained for mortality and reproduction of 10-12 or 20-22 days old springtails were compared. For CYP, the EC_{50} ($122 \text{ mg kg}^{-1}\text{soil}_{\text{dw}}$) for the effects on reproduction was significantly lower for the older compared to the juvenile springtails (EC_{50} of $241 \text{ mg kg}^{-1}\text{soil}_{\text{dw}}$). For TEF, the LC_{50} for the younger springtails was significantly lower ($0.51 \text{ mg kg}^{-1}\text{soil}_{\text{dw}}$) compared to that for the older animals ($0.91 \text{ mg kg}^{-1}\text{soil}_{\text{dw}}$). Even though the reproduction of both life stages was significantly inhibited by TEF, no significant difference was observed between different ages. These results show that the life stage of *F. candida* at the beginning of the toxicity tests can significantly influence the results obtained. Additionally, the influence of animal age on the test outcome was partially dependent on the active substance tested. This life-stage dependent effect should be considered in future studies to understand how pesticides can influence the role of SPU.

1.01.P-Mo016 Untargeted Lipidomics to Assess the Response of the Non-Targeted Species *Folsomia candida* to Sub-Lethal Concentrations of Teflubenzuron

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Increased rates of pesticide production brought serious concerns regarding their impact on non-targeted organisms in the environment. Soil organisms as Collembola (or springtails) widely contribute to soil fertility and the nutrient cycle in ecosystems. Therefore, springtails are an important model for ecotoxicological risk assessment (ERA). Classical approaches as reproduction, growth, and mortality are the main test systems used in ERA. Molecular “omics” studies have become an important part of ERA to study the mode of action of pesticides for non-target species. Untargeted screening of molecular changes can provide a more detailed picture of the sub-lethal effects of chemicals present in low concentrations in the environment. In this work, untargeted lipidomics was applied to study the potential toxicity effects of Teflubenzuron on the springtails *Folsomia candida*. Springtails were exposed (7 days) to three environmentally relevant concentrations (0.0056, 0.0139, and 0.0348 mg/kg soil) of Teflubenzuron. Teflubenzuron showed a wide range of significant alterations in the lipid profile. Mainly phospholipids (PC and PE), triglycerols (TG), and fatty acids (FA) were affected. These lipids are primarily involved in membrane synthesis, lipid storage and energy metabolism. Teflubenzuron exposure showed an increase of oxidised forms of TG and FA in springtails which can be indicators of oxidative stress. Moreover, the increase of oxidised polyunsaturated FA (PUFA), shown on all levels of exposure, may cause severe problems since PUFA play a vital role in organisms' development. The decrease of TG can probably be linked to stored lipids which were used as an additional energy source to cope with stress. The decrease in TG in combination with the upregulation of monounsaturated FA and sterol esters indicates the balancing of lipid storage in the body. Dysregulation of PC and PE lipids, associated with the endoplasmic reticulum (ER) and mitochondria, can indicate ER stress. ER is closely connected with lipid droplets synthesis and regulation. Alteration of PE can indicate the disruption of the mitochondria membrane and could further lead to oxidative stress and potential malfunction of glucose metabolism. Hence, a combined study of lipidomics and classical ERA approaches can contribute to a better understanding of the toxicity of Teflubenzuron from the molecular to the population level.

1.01.P-Mo017 Older Age Reduces the Sensitivity of *Daphnia magna* Neonates to the Chitin Synthesis Inhibitor Teflubenzuron

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Chitin is a major component of the arthropod exoskeleton and its synthesis is an essential process for arthropod development. It contributes to development by facilitating periodical shedding of the exoskeleton and replacing it with a new one (also called molting). Both, chitin synthesis and molting, are ultimately orchestrated by the hormone 20-hydroxyecdysone. As these processes are conserved in arthropods, they can be targeted to develop strategies for arthropod pest management. One of the substances marketed for this purpose is teflubenzuron, an arthropod growth regulator from the compound class of benzoylphenylureas. As teflubenzuron interferes with chitin synthesis, an endocrine controlled process that occurs periodically, we hypothesized that the age at the time of exposure is a determining factor for the sensitivity of neonatal *Daphnia magna* to the compound. We therefore exposed *D. magna* neonates of different age windows (0-4 h, 4-8 h, 8-12h, 0-12h and 0-24h) to different concentrations of teflubenzuron (0.1, 0.5, 1, 2, 4 and 6 µg/L) for 48 h and monitored molting and survival after 24, 36 and 48 h of exposure. Our results show that the endpoint of molting is generally accompanied by high variability within the data, even with narrow synchronization windows (0-4h). Analysis of dose response curves (comparison of 48h EC50s) revealed that younger *D. magna* neonates are more sensitive to teflubenzuron than older ones, where the youngest animals (0-4 h old) are three times more susceptible to teflubenzuron than the oldest (8-12 h old), and about one order of magnitude more susceptible than animals synchronized according to OECD test guideline 202 (0-24h old). Together, we demonstrate the importance of age in acute *D. magna* tests with substances interfering with chitin synthesis. Our results might have implications in the development and refinement of testing strategies for substances interfering with chitin synthesis in arthropods. Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 859891 and supported by NIVA's Computational Toxicology Program, NCTP (www.niva.no/nctp).

1.01.P-Mo018 Physiological Colour Change in Cuttlefish Skin Explants As an Innovative Tool for Contaminant Risk Assessment

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Colour change is a widespread ability among animals known to fulfil a range of biological functions: thermoregulation, UV-protection, crypsis or even communication. It can be achieved either by the production, degradation or chemical modification of pigmented structures (*i.e.*, morphological colour change) or by changes in their intracellular distribution (*i.e.* physiological colour change). Depending on the taxa, the physiological colour change mechanisms can be controlled by nervous and/or hormonal pathways, with which many environmental contaminants can potentially interfere. Hence, quantitative approaches of physiological colour change may provide new opportunities for toxicological risk assessment. Cephalopods provide well studied models for their colour change abilities. Their skin is a complex organ that includes a variety of chromatophores (yellow-, red-, and dark-pigmented cells) laying on top of light-reflecting leucophores and iridophores. Dynamic colour changes in cephalopods mainly involve the mechanical action of chromatophore-associated neuromuscular structures, resulting in the dispersion or concentration of pigmented organelles. Thus, we have developed an *in vitro* bioassay combining the preparation of skin explants from the common cuttlefish (*Sepia officinalis*) with a standardised, reproducible protocol of image acquisition and a software able to quantify the chromatophore activity in response to the topical application of contaminants. To address the specific problem of detecting and individualising overlapping chromatophores that the existing methods based on segmentation do not solve, we developed an algorithm based on deep learning. Its performances are illustrated here by the analysis of responses to selective serotonin reuptake inhibitors (SSRI-antidepressants) and putative endocrine disrupting chemicals.

1.01.P-Mo019 Application of Molecular Tools to Update Aquatic Ecotoxicology Monitoring

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The field of aquatic ecotoxicology has developed validated gold standard methods to identify, assess and reduce relevant contamination in aquatic ecosystems (Schmitt-Jansen et al. 2008). Both zooplankton and phytoplankton are used in ecotoxicological studies as they are bioindicators of water quality (Parmar et al. 2016) and anthropogenic stressors can adversely impact community assemblages and ecosystem function. To date, the study of freshwater planktonic organisms has been challenging as taxonomic identification requirements are frequently expensive, time-consuming and often require a specialist in the field (Elias-Gutierrez et al. 2018). Using molecular techniques, plankton identification is becoming easier and more accurate (Djurhuus et al. 2018). The overall objective of this project is to apply metabarcoding techniques to both identify and quantify the relative abundance of indicator-genera within in-field ecotoxicity studies, to replace or complement traditional microscopy techniques. Firstly, a database was created by retrieving all the available sequences in NCBI GenBank and BOLD for selected zooplankton and phytoplankton genera. Here we present a comparative analysis of molecular (sequence numbers) and morphological (abundance counts) data, focusing on nine taxonomic groups, to show baseline variation in the zooplankton community throughout the season. The 18S hypervariable region was sequenced for 35 zooplankton samples collected from five different mesocosms at CEA, Boxworth between May and September 2020. The morphological data of the seasonal mesocosms samples showed a seasonal variation of the nine most abundant genera. When compared to a previous study conducted on 12 mesocosms at the same site (Brooks et al. 2019), a similar seasonal pattern for the majority of genera was found. The molecular

results are expected to support the hypothesis that DNA metabarcoding can augment the morphological identification for estimating the diversity and composition of the zooplankton assemblage.

1.01.P-Mo020 Spatio-Temporal Patterns in the Gene Expression of the Copepod *Temora longicornis* in Response to Chemical Pollution

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Due to their rapid responses to environmental variation, planktonic organisms are used as bio-indicators of ecosystem changes. With the need for better understanding the impact of a changing environment on zooplankton communities, zooplankton monitoring programs have been carried out in the marine environment globally since the early 20th century. Most zooplankton monitoring studies focus mainly on variability in biodiversity and biomass. However, this approach is hindered by challenges in the identification, which is time-consuming, complicated and requires biological expertise. Advances in practical, cost-effective molecular approaches, such as (meta)barcoding, helped overcome the issues with morphology-based biomonitoring. Yet, a more comprehensive molecular data set would be able to identify and assess the impact of the main drivers of changes in the marine ecosystem, rather than only determining species richness. Since responses to environmental stress are initially genome-driven, a genetic understanding on the physiological responses to stress can help predict potential responses to a changing environment in the future. In this project, we focus on the potential effects of various environmental stressors (changes in temperature, salinity and concentration of PCBs and PAHs as a proxy for chemical pollution) on the gene expression of the calanoid copepod species, *Temora longicornis*, the dominant zooplankton species of the southern part of the North Sea. Therefore, this study investigated transcriptome-level profiles of adult *T. longicornis* that were collected at four stations in the Belgian part of the North Sea (BPNS) at different time points in a four year sampling campaign. Zooplankton samples were collected with the research vessel (RV) Simon Stevin on 35 (bi)monthly sampling campaigns in 2018 till 2021 (start: 20th February 2018, end: 22d of December 2021). From the obtained data, we aimed to identify the most active metabolic pathways and we tried to place these results into a broader context of physiological activities. Next, we constructed gene-co-expression networks, identified hub genes and we tried to obtain a mathematical relationship between these networks/hub genes and (1) the measured environmental variables and (2) phenotypic characteristics of interest (i.e. densities and biomass), defined by a generalized additive model. As such, we aim to identify molecular endpoints that can be consistently anchored to phenotypic changes under multi-stress conditions and at the same account for potential biological variability.

1.01.P-Mo021 Standardized and Reproducible Quantitative Approaches to Screen Antibiotics and Organophosphates Pesticides Effects on Freshwater Planarian: Setting the Stage for High-Throughput Screening in (Eco) Toxicology

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For the two last decades, the number of toxicological studies using freshwater planarian have been steadily increasing, showing the planarian to be a really promising alternative model to assess pollutant that may ends up in aquatic environment. Planarian have been suggested as a bioindicator of the quality of freshwater habitat. Freshwater planarians are invertebrate flatworm worldwide distributed, easy to collect, and to maintain in lab. Their small size allows medium to high-throughput screening of toxicity on a whole-animal system. Planarian can enable the assessment of toxicity of pollutant on many levels: 1) they are secondary consumers feeding on other aquatic invertebrates, planarians are good indicators of chemical risk on the aquatic ecosystem; 2) the planarian nervous system share the same neuronal subpopulations and neurotransmitters as the mammalian brain, they are potential screening tools for predicting pollutant toxicity for mammals. In this study, we design a standardized, reproducible quantitative protocol to evaluate the effect of two chemicals used in agriculture: gentamicin, a broad-spectrum antibiotic, and an organophosphate pesticide, chlorpyrifos through multiple approaches. Planarian were screened for mortality at 7 concentrations of chlorpyrifos over a 24 h period. Effects of sublethal concentrations of chlorpyrifos and 3 concentrations of gentamicin, were screened through quantitative assessment of multiple behaviours. Planarian responses were evaluated with 3 behavioural challenges: exploratory behaviour, response to light stress and negative phototaxis. These tests have shown to be reliable and repeatable for toxicological screening in lab.

1.01.P-Mo022 Variations in the mRNA Expression After Short- and Long-Term Exposure to Metformin on Adult Marine Mussels

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The widely prescribed antidiabetic drug metformin has the potential to accumulate and persist in water bodies, with little-known consequences for aquatic biota. Here, we report the biological responses of a sentinel organism, the blue mussel *Mytilus edulis*, exposed to a range of concentrations of metformin found in the aquatic environment, and also exceeding those levels, representing a worst-case scenario. The effects on several target genes in mussels' gonads are discussed following short- and long-term exposure to metformin. Metformin induced variations in the mRNA expression of all transcripts investigated here, with some transcripts (*VTG*, *V9*, *CASP8*, *FAS*) displaying fluctuating results. Several transcripts followed concentration-dependent trends, mostly in the short-term exposure, as shown by the expression of *ER2* and *HSP70*, whilst *BCL2* expression also showed a dose-dependent result after long-term exposure. The monotonic responses exhibited by some transcripts indicate their potential as biomarkers of metformin exposure. The remarkable variation in expression also observed between short- versus long-term exposures reflects the importance of contact time between metformin and the organism in the natural environment, as shown by all transcripts.

1.01.P-Mo023 Investigating the Background Individual Metabolomic Variability of the Freshwater Invertebrate, *Gammarus pulex*

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The (pseudo)persistence of emerging contaminants and other organic micropollutants in the environment is a cause for concern among the organisms that are exposed to them. Recently, 'omics technologies are increasing in their application within environmental toxicology to understand the effects of these exposure scenarios in both lab and field collected organisms. However, metabolomics is an emerging field and to interpret the use of metabolite data in the understanding of toxicological responses is challenging. The variability in individual metabolomes for a species, or a "background metabolome" should be established to determine possible confounding factors such as age, biological sex and moulting (among others) that may influence data interpretation. Furthermore, for field-collected invertebrates previous exposure history could be an important consideration when using them in environmental risk assessment. Thus, we have characterised the effect of these factors on the metabolic variability in the freshwater invertebrate, *G. pulex*. Herein, an analytical method is presented for the extraction and non-target analysis¹ of the metabolome in *G. pulex*. Briefly, a dual phase liquid extraction was used followed by HILIC-HRMS to enable detection and annotation of metabolic features extracted from individual animals (~5 mg dw). Animals collected from the field were analysed immediately and compared to animals that were extracted after a set period of acclimatisation to laboratory conditions. The variability of metabolomes was analysed using XCMS and MetaboAnalyst. The results indicated that sex, moulting stage and acclimatisation period affected the metabolic variability and factors that are likely to influence metabolomic analyses should be investigated to aid understanding of pathways involved in effect-based studies, especially where these approaches might be used to develop adverse outcome pathways. Furthermore, it may be prudent to pre-select animals based on these confounding factors to reduce noise in the data. Overall, the characterisation of metabolic variance for invertebrates along with the use of metabolomics shows a very powerful approach for understanding adverse effects that may be associated with environmental contaminants.

1.01.P-Mo024 Neurotransmitter Systems in Marine Invertebrates and Their Potential Perturbation by Environmental Pollutants

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Neurotransmitter systems are major elements in the transmission of information between the central nervous system and target organs for the regulation of physiological functions. These systems are highly conserved in the animal kingdom. Therefore, neurotoxic compounds could interact with the same targets in vertebrates as invertebrates. In some invertebrates, monoaminergic neurotransmitters (serotonin, dopamine, norepinephrine, octopamine) can influence neurohormones. Thus, a modulation of these systems by environmental pollutants may induce endocrine disruption. Such endocrine disruptive effects remain sparsely studied in marine invertebrates. To shed light on neuro-endocrine perturbation, it is necessary to better understand the functioning of these monoaminergic systems in marine invertebrates. We here present a comparative approach including two species covering two important phyla of marine animals: the green crab *Carcinus maenas*, a crustacean, and the cuttlefish *Sepia officinalis*, a mollusk. The different monoamines in the nervous systems of both species are mapped using immunohistochemistry and quantified by LC-MS/MS following the exposure to putative endocrine disrupting compounds.

1.01.P-Mo025 Planaria As an Alternative Approach to Animal Testing in the Future

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The adoption of the 3R rules (reduce, refine and replace) encourages to limit the use of vertebrate animals in scientific research and aims at developing new model organisms. Invertebrates become increasingly more popular in order to replace vertebrates. Their relatively high sensitivity to pollutants makes them promising as screening tools to predict the toxicity of various molecules in vertebrates including mammals. Planaria is an invertebrate flatworm living in freshwater, worldwide. It's well known for its regenerative capacities, and low maintenance requirements. Planaria are endemic invertebrate organisms, easy to collect locally, kept in controlled conditions, and are not subject to regulation in terms of animal experimentation. Planaria is emerging today as an *in-vivo* model for (eco) toxicological study. Planaria has a sensitivity comparable to other well-established freshwater invertebrate models such as daphnia but with some additional advantages. Planaria can regenerate itself and possess a nervous system with all the molecular characteristics of higher vertebrates such as central nervous system and neurotransmitters. The standardization of experimental practices is crucial for the consistency and comparison of data between laboratories. Four planarian species are commonly used for toxicological studies but have different sensitivities to the same chemicals. The aim of this presentation is to highlight advantages of using planaria as an (eco)-toxicological model. Three steps are presented: First by implementing data from literature on using planaria as model, secondly by proposing a standardized protocol in behavioral study and third by validating the use of the planaria as an (eco)-toxicological model.

1.01.P-Mo026 Caenorhabditis elegans Reproduction As a Chronic Endpoint for Fast Screening in Ecotoxicology

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In vitro assays are fast and high throughput tests that also reduce the need for vertebrate testing. However, there is still need for whole-animal testing that can i) capture wide-arrays of toxicity mechanisms and ii) show how a chemical behaves in a

compartmentalized model. The nematode *Caenorhabditis elegans* is a widely used model to assess toxicity of sediment, soil, pore water and aqueous samples. Considering its small size (up to two mm in length) and short life-cycle, *C. elegans* could be an especially useful whole-organism with a chronic endpoint (i.e., reproduction) for high-throughput tests. The aim of this work was to explore different measures of *C. elegans*' reproduction output such as offspring number, offspring size and biomass (offspring size × number) and evaluate its sensitivity towards common environmental contaminants and stressors compared to other commonly used model organisms. *C. elegans* reproduction tests are carried out by exposing pre-pregnant adults to the stressor of interest for over 72 hours and recording the number of offspring produced by a single adult. We additionally registered the number and size of offspring and total biomass with the aid of image processing software and compared their responses to the standard toxicant benzalkonium chloride (BAC). To evaluate the sensitivity of *C. elegans* towards different common environmental contaminants, we used the list of 12 organic water pollutants of different modes of action carefully curated by Altenburger and colleagues (2018). Our results indicate that biomass might be a more sensitive and comprehensive endpoint for *C. elegans* reproduction when compared to number of offspring per reproducing adult, capable of capturing different modes of action (i.e., both number and size of offspring). In addition, although *C. elegans* does align with other widely used whole-organism assays such as daphnia and zebrafish, it is, however, on average one order of magnitude less sensitive despite the endpoint being reproduction and therefore hypothesised to be more sensitive than survival.

1.01.P-Mo027 Integrated Biomarker Response in Signal Crayfish *Pacifastacus Leniusculus* Exposed to Diphenhydramine
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Pharmaceuticals have been repeatedly detected as emerging contaminants, particularly in streams receiving a large amount of treated wastewater. Their continuous discharge, relative stability in the environment, and bioaccumulation in living organisms are the main predictors of potential ecotoxicity effects on non-target aquatic biota. Diphenhydramine (DPH) is a pharmaceutical with multiple mode of action, primarily designed as the antiallergy therapeutic drug. Among antihistamines, this drug is a significant contaminant repeatedly detected in surface waters. Signal crayfish (*Pacifastacus leniusculus*) was selected in our study as a model organism due to its' ecosystem importance, rapid growth, large body size, and easy culture. We investigated a subchronic effect of low (2 µg/L), environmental (20 µg/L), and elevated concentration (200 µg/L) of diphenhydramine on crayfish. Antioxidant enzyme activities, lipid peroxidation, and acetylcholinesterase activity were assessed as toxicological biomarkers. Integrated biomarker response indicated the highest drug-induced toxicity effects on crayfish gill and muscles at elevated diphenhydramine concentration. In contrast, the highest effect was observed in the hepatopancreas after exposure to environmental concentration. Diphenhydramine did not significantly affect acetylcholinesterase activity under any tested drug concentration. We conclude that the integrated biomarker approach is an effective tool for evaluating the toxicity effects of pharmaceuticals on aquatic invertebrates. The research was supported by the Ministry of Education, Youth and Sports of the Czech Republic – projects "CENAKVA" [LM2018099], and CZ.02.1.01/0.0/0.0/16_019/0000869 PROFISH.

1.01.P-Mo028 Successful Development of a Test Protocol for the Dung Beetle *Onthophagus Taurus* for Usage in the Environmental Risk Assessment of Veterinary Pharmaceuticals (VMPs)

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For more than two decades the environmental risk of veterinary pharmaceuticals (VMPs) is assessed before these products can be marketed in the EU. This is especially true for parasiticides because of their direct entry into the environment and their biocidal mode-of-action. Once in the environment, these VMPs can impact ecosystem functions, in particular the decomposition of dung, and also dung organism biodiversity. A taxon that reaches high abundances in dung pads and that is considered as threatened under the current worldwide insect decline are dung-breeding beetles. The OECD guidance 122 for testing of dung beetles (OECD 2010) provides a protocol using the larvae of *Aphodius constans*. This species is winter-active and endures the summer in a resting stage. It became evident that it is hardly possible to culture this species under lab conditions. Consequently, beetles have to be caught in the field which is only possible from December to April. A further shortcoming of the current test protocol is that only larval mortality is taken into account. Over the past decade, a test protocol with *Onthophagus taurus* was developed and ring-tested. After several improvements, this species can now be cultured in the lab and thus, tests including reproductive endpoints can be conducted all the year round. Currently, the test system is running reliably and results using Ivermectin as reference item are reproducible. Moreover, several studies under GLP have already been conducted in different laboratories and their results have been used in the Environmental Risk Assessment of VMPs. Here, the test set-up is described and results of the final ring-test and control and reference item data from GLP studies are presented. The authors propose that the test protocol for *Onthophagus taurus* should be integrated into the existing OECD guidance document 122.

1.01.P-Mo029 Comparing the Sensitivity of Field Collected Lentic and Lotic Mayfly Species After Chronic Exposure to the Insecticide Fipronil in the Laboratory

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Aquatic insects are often exposed to environmental pollutants, e.g. plant protection products. Especially aquatic mayfly larvae are frequently exposed and show high sensitivities against such contaminants. For the risk assessment, often standardized test systems

with *Daphnia* sp. are used but can cause an underestimation of the risk since they are less sensitive. Thus, in Europe we are still lacking standardized test systems with more sensitive species like mayflies, particular for chronic tests. At Fraunhofer IME, two test systems for chronic toxicity tests with mayfly species are performed. In both tests, the insecticide Fipronil is used as test substance with concentrations of 0.037, 0.075, 0.15, 0.3 and 0.6 µg/L. Field collected larvae in early development stages are used in both tests. During the tests, the grazing larvae are fed with diatoms of *Navicula* sp. grown on small tiles. Test solutions and food tiles are replaced twice a week. In the first test, a chronic test system for the lentic mayfly *Cloeon dipterum* is used. As endpoints the development throughout the test, emergence, immobility and mortality of the larvae are examined. The test is performed in glass beakers filled with 500 mL test solution under conditions of standing water until emergence of all introduced larvae. The development stages of each larvae are determined twice per week until all larvae have emerged or died. Emerged individuals are sampled for further transcriptomic analysis. In the second study, a chronic toxicity test with lotic mayfly larvae of the family Heptageniidae is performed under stream conditions. The individuals are placed individually in small cages that circulate through a test unit thereby creating a specific flow of around 0.1 m/s in each cage. The larvae are exposed for at least 21 days. The endpoints immobility and mortality are determined twice a week. The body length of each animal is measured prior test start and again at the end of the study to determine the endpoint of larval growth. Both tests were already started. First results of the test with *C. dipterum* show the trend of a reduction in the development rate of the larvae exposed to the two highest concentrations on day 20. An increase of mortality with larval growth is observed. The entire results of the ecotoxicological tests and the transcriptomics of *C. dipterum* will be presented.

1.01.P-Mo030 The Estuarine Bivalve *Scrobicularia plana* As a Model Species for the Assessment of Chemical Stress Caused by an Ubiquitous Flame Retardant

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Tris(2-butoxyethyl)phosphate (TBOEP) is a flame retardant widely used in most industries. Its occurrence in aquatic environments is vast, having been detected in several matrices worldwide, including Portuguese estuaries. TBOEP produces toxicity to aquatic species through oxidative stress and endocrine disruption. Still, investigation is scarce and toxicity assays are limited to standard freshwater species cultivated under laboratory conditions. There is a lack of information about chemical stress in marine environments, as well as studies using organisms collected in field. *Scrobicularia plana* is a bivalve species from brackish waters. This invertebrate is proposed as a model for ecotoxicological assays due to its sensitiveness and easy handling, besides its great abundance in intertidal soft-substrate waterbodies of NE Atlantic from Norway to the Mediterranean and West African regions. This work evaluates TBOEP's toxicity in *S. plana* collected at the Mondego estuary in Figueira da Foz (Portugal), in terms of behaviour and molecular responses. The biomarkers selected are the activity of glutathione-S-transferase (GST), the thiobarbituric acid reactive substances (TBARS) and the protein content. Endpoints were assessed in organisms after field collection, after acclimation to lab conditions (20 psu filtrated water, constant aeration, 12h^L:12h^P, 20±2°C) and after TBOEP exposure, to avert confounding factors when linking the biomarkers' responses to the contamination. The experimental design consisted in 96h of exposure to TBOEP (0.1, 1 and 10 mg/L). In terms of behaviour, more syphon activity and less reaction to the stimulus were observed at the exposures. GST activities remained unaffected by the exposures and by the settled lab conditions when compared with the field. Regarding TBARS, lower values under lab conditions than in the field were observed, due to its high sensitiveness. The presence of TBOEP increased the levels of TBARS quantified when compared with the control, suggesting the occurrence of lipid peroxidation. Further investigation is needed to confirm a potential defense mechanism involved, thus, biochemical profiles are being analyzed. The species, endpoints and experimental design here proposed contribute to knowledge of toxicity assessment of specific chemical stress by identifying suitable biomarkers. Nonetheless, further studies with new endpoints will provide a better understanding of TBOEP's toxicity in marine environments.

1.01.P-Mo031 Automatic Counting of *Artemia* Nauplii and Cysts - Applications in Marine Ecotoxicology

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The ecotoxicological evaluation of the chronic effects of chemicals in the reproduction of marine species commonly includes a counting procedure of the progeny of the exposed individuals in early development stages, e.g. nauplii. Considering marine model species, the counting of nauplii in ecotoxicology bioassays is very difficult to perform, due to the small size and also due to the erratic movements of the nauplii. *Artemia* spp. have been widely used as a biological model in toxicity testing bioassay due to the cost-efficiency and its cysts high adaptabilities to harsh under laboratory conditions, being acute mortality and acute cyst hatchability, two of the three sensitive endpoints commonly used; thus, it would be highly valuable to perform the counting automatically. This study aims to assess the performance of a computer-based technology (Patent US10591401, Patent CA2965273C, Pending patent EP3214426A1: Oliveira e Silva et al. 2020) on counting *Artemia salina* and *A. franciscana* nauplii and cysts, by comparing the automatic counts with the results of manual countings. At least 55 comparing runs (manual vs computer) were performed for each species, having from n=0 up to n=200 nauplii in the vial (50mL solution). The procedure was repeated considering *A. salina* cysts, with 10 runs, having from n=0 up to n=100 cysts in the vial (50mL solution). The comparison of the automatic vs manual counts of *Artemia* spp. nauplii and cysts show that the automatic counts of nauplii and cysts showed a 99% accuracy and a relative standard deviation of 1.7%, being y=1.00x with r²>0.999 linear correlation. These results show that the computer-based counting device accurately counted the organisms, thus showing its applicability as a replacement of manual counting. This tool might also be applicable to assess acute cyst hatchability, starting by quantifying the

number of total cysts to be submitted to the bioassay, and later by quantifying the subsequent hatched organisms (nauplii) under test. This computer-based counting might be extended to other marine organisms of similar size, thus facilitating reproduction, hatchability, and life-cycle bioassays in marine ecotoxicology.

1.01.P-Mo032 Comparative Study of Three Soil Arthropods Extraction Method (MacFadyen; Tullgren Funnel (Burkard); Modified Berlese-Tullgren) in South of France

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Soil micro-arthropods, such as mites and springtails, are largely represented in most soils. They play an important role on decomposition processes and are important reservoirs of biodiversity in ecosystems. The potential impact of plant protection products on the structure of the soil micro-arthropod communities can be assessed in dedicated studies or as part of larger arthropod studies focused on the different arthropod habitats, including soil, by different methods. The aim of this study is to compare different soil arthropod extraction methods. The soil micro-arthropods were sampled with soil cores and extracted for identification with MacFadyen, Tullgren funnel or the modified Berlese-Tullgren methods. The extraction efficiency of these 3 methods were compared by the number of specimens and the diversity per sampling method and per unit of soil sampled. Soil cores were sampled simultaneously in 12 locations in a meadow under the same conditions such as soil moisture and temperature. 6 intact cores of 5cm diameter and 5cm depth were collected per location for MacFadyen extraction, 2 intact cores of 11 cm diameter and 5cm depth for Burkard extraction and 5 cores of 8cm diameter and 5cm depth, with the soil mixed and standardized to 1kg sample, for the modified Berlese-Tullgren setup. With this experiment, the Burkard method showed a statistically significant higher extraction efficiency in terms of abundance of specimens and diversity of Collembola than the MacFadyen method when 2 cores per location are taken for Burkard extraction and 6 cores per location are taken per MacFadyen extraction based on the recommendations of the manufacturers and guidelines. The modified Berlese-Tullgren with mixed soil showed a statistically significant lower extraction efficiency for Acari and Collembola compared to MacFadyen and Burkard methods. On the other hand, regarding extraction efficiency standardized by 500g of soil, the extraction of the MacFadyen method was more efficient for Poduromorpha collembolans, with statistically significant higher numbers in comparison to Burkard and modified Berlese-Tullgren and more efficient for the total number of Collembola than the modified Berlese-Tullgren. The observed diversity of collembolans/500g soil in the MacFadyen method was not statistically significantly different from the Burkard method, but both showed statistically significantly higher diversity of arthropods extracted than the modified Berlese-Tullgren method.

1.01 Advantages of using lab and field collected invertebrates in ecotoxicology: Challenges and opportunities for Environmental Risk Assessment (Virtual Only)

1.01.V-01 Assessment of the Response of *Artemia franciscana* Exposed to Detergents

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Detergents are widely used in daily personal care and household cleaning products, as well as in a variety of industrial applications. Large quantities of these products are discharged into wastewater that reaches treatment plants or directly into the aquatic environment in areas where there is no wastewater treatment. Because in our country the studies with detergents are scarce in this paper was evaluated the toxicity of these compounds on *Artemia franciscana*. Static tests were carried out with duration of 96 hours. 80 organisms were exposed to sublethal concentration (1 mg/L) of different types of detergent (2 for general use, 1 biological product and 2 dishwashers) plus a control without toxic. Every 24 hours was determined the stress degree in the organism estimating the O:N index, also was determined the level of lipid peroxidation, evaluating the Tbars. Significant differences were observed between the control response and the organisms exposed to detergents ($p < 0.05$). The O:N ratio had a value above 20 which indicates that the organisms were not in a state of stress, after 24 hrs of exposure, except for the test with the dishwasher detergent. A significant decrease of this index was observed at 72 hrs in all tests with values less than 9, a fact which indicates that the organisms were in a high stress state. MDA levels were higher in organisms exposed to dishwasher detergent (145.4 to 213 nM MDA/g), the value obtained in the control was 2.4 nM MDA/g Because the wastewater treatment is limited and detergents are discharged directly into the environment is important to know the potential adverse effects of these compounds to propose mitigation measures.

1.01.V-02 Biomarkers Evaluation on *Crassostrea Palmula* in La Paz Bay BCS , Mexico

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The native oyster *Crassostrea palmula* has permanent populations along La Paz Bay. We used the species to determine their response to xenobiotics with an evaluation of 3 biomarkers: oxidative stress (Tbars), AchE inhibition and genetic damage (micronucleus frequency). The studies were carried out in samples of the gill tissue obtained from oysters in three localities, two related to city activities and the other away from city influence, to detect the presence of toxic and genotoxic substances, and to evaluate the use of this biomarkers as a reliable tool in environmental biomonitoring studies. Thirty adult oysters (5.15 ± 0.58 cm) were collected in winter (February 2018) and summer (August 2018) in each locality. The results indicated that there are significant differences between the sites. The biomarkers presented values similar to the control (oysters kept under controlled conditions in the laboratory), in the oyster samples collected in the area without influence of the city in comparison with those obtained in organisms collected in urban-influenced areas. The results agree with the pollutants levels registered in the place where the oysters were collected. This biomarker seems to be a good tool in environmental biomonitoring studies.

1.01.V-03 Comparative analysis of CMIT/MIT toxicity across *Daphnia pulex* strains using epigenetics and proteomics

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The genome and epigenome of an organism respond to a variety of environmental cues and can alter physiological responses in order to adapt to the surrounding environment. Consequently, the adaptation process, whether genetically and/or epigenetically derived, is an important consideration in evaluating the adverse outcomes of toxicants since it has the potential to change the sensitivity and tolerance of organisms to certain stressors. In this study, we aimed to identify the difference in sensitivity and epigenetic response to chemicals using different field-collected *Daphnia* populations which were adapted to different habitats. To this end, comparative toxicity to the mixture of 5-Chloro-2-methyl-3(2H)-isothiazolone and 2-methyl-3(2H)-isothiazolone (CMIT/MIT), which is an isothiazolinone biocide broadly used in various types of consumer products, was carried out by investigating the alterations in mortality, reproduction, physiological traits, global DNA methylation and proteome. Results showed that intra-species difference in sensitivity (*Daphnia pulex* from Rennes (DPR) vs. *Daphnia pulex* from Alsace (DPA)) is greater than inter-species difference (*Daphnia magna* from Rennes (DMR) vs. *Daphnia pulex* from Rennes (DPR)). DPR has larger body size, higher heart rate and higher sensitivity to CMIT/MIT than DPA. Interestingly, the trend of alteration in global DNA methylation level differed between the two populations. Proteomic analysis also supported that DPR and DPA-specific protein alteration and the associated pathways can cause the observed difference in susceptibility and epigenetic response to CMIT/MIT. Therefore, intra-species variation should be considered in evaluation of ecotoxicity to biocide chemicals since the sensitivity to chemicals varies depending on the environment where organisms are adapted. Additionally, epigenetic programming might be altered by environmental factors and can contribute to the observed differences in sensitivity to chemicals.

1.01.V-04 Comprehensive Assessment of WATER Quality Based on Physiological State Evaluation of Indigenous Freshwater Mussel (*Unio pictorum*) and Heavy Metal Contents in 3 Matrices: CASE Study on the Transborder Narva River Russia-Estonia

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The sustainable existence of aquatic ecosystems implies a stable state of water bodies with high water quality, their plant and animal components. Bivalve mollusks are the main and numerous representatives of the biota of fresh waterbodies of the Leningrad Region, which is actively involved in maintaining the equilibrium state of the ecosystem, as well as in self-purification processes. The purpose of the study was to conduct a comprehensive study of the ecological state of the coastal water area of the Narva border river (Russia-Estonia, the river bank 3 km from the city of Ivangorod, Kingisepp district of the Leningrad region). Using an automated bioelectronic system of non-invasive cardiac activity monitoring, the background characteristics of the heart beatings of bivalves (*Unio pictorum*) from the Narva River were studied, the time of cardiorythm recovery after a short-term functional load (change in salinity to 6 ‰ within 1 hour) was calculated, indicating the good adaptive potential of the mollusk cardiosystem. In soft mussel's tissues, the contents of heavy metals (HMs, in particular Cd, Ni, Cu, Zn and Pb) were determined. MPI and BCF were calculated for each metal. Data (2019 and 2020) of the Nevsky-Ladoga Basin Administration of the Russian Federation FSBI "North-West Administration for Hydrometeorology and Environmental Monitoring" on the content of HMs in the surface water of the Narva River in this river target, closest to the sampling site, were also used, indicating good quality of the surface waters. The results showed good physiological state of mollusks, low content of HMs both in mussel's tissues and in sediments, as well as in the surface water of the Narva River in studied site. The results seems to be useful for the developing of methods for integrated assessment of the health of aquatic ecosystems in transborder areas.

1.01.V-05 Effects of Bisphenol a on the Growth and Reproduction of 4 Species of Cladocerans

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Bisphenol A (BPA) is a compound present in plastics type 3 and 7 that are used to make water bottles, baby bottles, disposable tableware, toys, food can liners and PVC products. This compound is harmful because it acts as endocrine disruptor. Its effects are known mainly in fish. The objective of this work was to evaluate the effects of Bisphenol A on the growth and reproduction of 4 species of cladocerans: *Daphnia magna*, *Ceriodaphnia dubia*, *Moina macrocopa* and *Chydorus* sp. Initially, an acute bioassay of 48 hours duration was carried out where 5 concentrations of BPA were tested, to determine the LC₅₀ (Lethal Concentration 50). Subsequently, a sublethal bioassay (21 days) was performed with 2 concentrations (LC₅₀/100, LC₅₀/10) where it was evaluated: time of the first molt, number of molts, time of the first spawning, number of spawning and growth rate. The LC₅₀ values obtained in the toxicity bioassays ranged from 5.4 ± 1.97 to 12.1 ± 3.8 mg/L, the most sensitive species was *Moina macrocopa*. Regarding the time in which the first molt occurred and the time of the first spawning, significant differences were observed between the organisms exposed to BPA and the control group (p > 0.05). An increase in time was observed for cladocerans to molt and have their first spawn. The number of spawning in organisms exposed to Bisphenol A was lower compared to the control group. In addition, a decrease in the growth rate was observed from 1.5% to 44.9%. From the results obtained we can conclude that BPA in sublethal concentrations affected the reproduction and growth of cladocerans, confirming that it has an endocrine disruptor effect on *Daphnia magna*, *Ceriodaphnia dubia*, *Moina macrocopa* and *Chydorus* sp.

1.01.V-06 Genotoxic Effect of Three Metals and Their Mixtures on Japanese Oyster *Crassostrea gigas*

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The Japanese oyster is an introduced species that grows in the Mexican Pacific coastal systems, because of its economic importance we performed an evaluation of the genotoxic effects of the metals: Cd, Cr and Pb and their mixtures on Japanese

oyster juvenile. Bioassays, with a duration of 72 hours, were carried out with 5 concentrations of each metal and their mixtures (1:1). Lethal concentration 50 (LC₅₀) were determined in the assays. Subsequently, a test lasting 10 days was carried out where the juvenile oysters were exposed to a sublethal concentration (LC1). At the end of the exposure period, the evaluation of lipoperoxidation (Tbars) and genetic damage (comet assay) in gill tissue was carried out. The toxicity of metals and their mixtures, based on the LC₅₀ calculated was (high to low effect): Cd > Cr > Pb. The most toxic mixtures were Cd + Cr and Cd + Cr + Pb. The degree of lipoperoxidation of gill tissues was high in oysters exposed to Cd and the mixture of the three metals. The Kruskal-Wallis test indicated that significant differences exist between the level of DNA damage in organisms exposed to different metals and the control group. The metal with major genotoxic effect was Cadmium followed by Chromium. Lead showed the lowest genotoxic effect. The mixtures with the highest genotoxic effect were Cd + Cr + Pb and Cd + Cr. It is important to continue research and monitoring to detect responses that indicate possible damage to oyster populations by the action of different tensors, to prevent irreversible deterioration of the stocks in the medium and long term.

1.01.V-07 Sublethal and Sub-Chronic Ecotoxicity of Five Synthetic Cathinones in Two Aquatic Organisms

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The abuse of psychoactive substances (PAS) has increased dramatically in the last years. PAS enter sewage systems and reach the wastewater treatment plants, which are not designed for their removal, leading to their frequent detection in effluents and aquatic ecosystems. Special attention has been given to synthetic cathinones (SCat), popular among consumers for recreational purposes. PAS are designed to change nervous system function, posing unpredictable adverse effects non-target organisms. Considering the scarce information available on SCat ecotoxicity, it is urgent to assess their toxicity on exposed organisms. This work aimed to study the toxicity of five SCat using two organisms of different trophic levels as model organisms: the microcrustacean *Daphnia magna* and the protozoan *Tetrahymena thermophila*. For that, a sublethal assay using daphniids was carried out at a single concentration of 10 µg/L to evaluate reproductive, biochemical, morphophysiological and behavioral effects. Additionally, a sub-chronic assay using the protozoan was carried out at concentrations ranging from 1.25 to 40 mg/L to evaluate effects on growth. Regarding microcrustacean, a generalized increase in the fecundity of the organisms was observed for all tested SCat although a significant increase was found for 3,4-dimethylmethcathinone (3,4-DMMC). On the other hand, 3-methylmethcathinone interfered with morphophysiological parameters, such as body size and heart rate. Concerning the protozoan, 3,4-DMMC also showed higher toxicity comparatively to the other SCat, causing a significant growth inhibition, while exposure to the remaining SCat did not affect growth. The present study showed low potential toxicity of SCat to the protozoan, except for 3,4-DDMC; in contrast, SCat interfered with reproductive and morphophysiological endpoints in *D. magna*. Further studies are ongoing to extend the current knowledge about SCat toxicity effects on these non-target aquatic organisms. **Acknowledgments:** This work was supported by national funds through FCT by means of the research project EnantioTox (PTDC/CTA-AMB/6686/2020) and the programmes UIDB/04423/2020 and UIDP/04423/2020. A. Pérez-Pereira acknowledges the PhD grant BD/CBAS/CESPU/04/2021.

1.02 Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future (Part I)

1.02.T-02 Exploring an Underlying Assumption of Baseline Toxicity QSARs for Fish Using a Mechanistic Bioaccumulation Model

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Baseline toxicity QSARs are well-established in the scientific literature and are useful tools for predicting acute toxicity in fish (LC₅₀s) or for including as part of the weight of evidence informing a Mode of Action (MOA) classification where empirical LC₅₀s are available for comparison. Because these QSARs are derived using equilibrium partitioning ratios (e.g., $1/LC_{50} = a \log K_{OW} + b$), they do not explicitly account for biotransformation of the chemical and its potential influence on the relationship between external concentration and internal body burden. The main objective of this study is to explore this key underlying assumption of typical baseline toxicity QSARs using a mechanistic bioaccumulation model (BIONIC v3.0) to relate external concentrations (LC₅₀s) to internal body burdens with and without considering biotransformation. To assess the extent to which the equilibrium assumption is approached, the BIONIC v3.0 model was applied to simulate bioconcentration factors (BCFs) and Critical Membrane Concentrations (CMC₅₀s) for a set of established nonpolar and polar “narcotics”. The simulations were parameterized based on test conditions, partitioning data and biotransformation rate constant estimates (k_B) generated using two available QSARs. The predicted BCFs are then compared to available empirical data ($n = 76$ observations). Predicted and empirical BCFs were highly correlated ($r^2 = 0.91$), there was no large systematic under- or overprediction and the Mean Absolute Error (MAE) corresponds to agreement within a factor of 2.5 on average. Broadly speaking, predicted CMC₅₀s for the case study chemicals based on the corresponding LC₅₀s are in the expected range (20–200 mmol/kg lipid). The predicted internal body burdens are often within a factor of two of the equilibrium (i.e., $k_B = 0$) values despite many chemicals exhibiting rapid biotransformation (i.e., predicted $k_B > 1/d$). The reason that BCFs are similar is that predicted gill elimination rate constants (k_W) are greater than biotransformation rate constants and accordingly the predicted BCF approaches $BCF = k_U / k_W$, which is consistent with equilibrium. This type of toxicokinetic behaviour (i.e., $BCF \sim k_U / k_W$ paradigm) underlies empirically-derived

baseline toxicity QSARs and is an important consideration when assessing other baseline toxicants which may not behave the same way with respect to the relative importance of gill elimination and biotransformation (i.e., $BCF \sim k_U/k_B$ paradigm).

1.02.T-03 Machine Learning As a Tool to Predict the Toxicity of Chemicals Across Taxa

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One of the pillars of modern civilization is the ability to synthesize and/or use an enormous range of chemicals, which allow for new and improved products and serve as pharmaceuticals, pesticides, food additives and the like. However, in order to know that use of these chemicals does not harm human- and ecosystem health, it is crucial to investigate the impact of these chemicals on those organisms potentially exposed. Usually this is done through in-vivo testing. Yet, beyond its obvious ethical implications, in-vivo testing is not scalable to large amounts of taxa and chemicals, because of its strong requirements in terms of time, money, and highly trained personnel. Machine learning comes in as a viable alternative with the potential of allowing us to explore the impact of large numbers of chemicals on many taxa: harmlessly, quickly and cheaply. We develop machine learning models that can quickly infer the mortality of a chemical on a certain fish species and highlight the importance of including information on both chemical and taxonomy as input features. We then analyze ways of comparing the reproducibility of in-vivo experiments with that of machine learning models trained on similar data, highlighting that these comparisons are generally unreliable, and propose to upper bound the reproducibility of in-vivo experiments, instead of trying to exactly estimate it. Finally, we introduce an operational way of showing whether *in-vivo* mortality can be well-captured by in-vitro assays on fish-cell lines, finding evidence that it indeed can. Thus, machine learning and *in-vitro* assays present themselves as valid alternatives to animal testing, if we will be able to assess their limitations when utilized in out-of-domain settings (e.g. exotic chemicals and species).

1.02.T-04 Reduced Transcriptome Arrays (EcoToxChips) to Derive Transcriptomic Points-Of-Departure (tPODs) for 17 α -Ethinylestradiol in Fathead Minnow Embryos

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Traditional toxicity testing is challenged because it relies on time-consuming and expensive exposure studies with live animals. New approach methods (NAMs) that utilize mechanistic information and alternative test systems show significant promise to address these challenges and support chemical hazard assessment. Recent studies suggest that benchmark doses (BMDs) derived from whole transcriptome experiments are reasonably predictive and protective of apical BMD. However, whole transcriptome analyses require sophisticated facilities and highly trained experts for data evaluation and interpretation, limiting accessibility of such approaches to the broader testing community. This study compared transcriptomic points-of-departure (tPODs) derived from whole and reduced (375 gene targets) transcriptomes of fathead minnow embryos exposed to 17 α -ethinylestradiol (EE2). RNA was extracted from pooled whole embryos after short-term exposure of non-protected life stages to graded concentrations of EE2, and then subjected to RNAseq and qPCR (EcoToxChip) analysis. BMD analyses yielded tPOD estimates that closely approximated apical PODs found in the literature. tPOD estimates were comparable between data sets generated with RNAseq and EcoToxChip analyses. However, the reduced gene set did not permit the estimation of the median of the 20 most sensitive gene BMDs (omicBMD20), which has been shown to be the most sensitive endpoint when deriving tPODs from RNAseq experiments. Both analysis types identified the same top dysregulated genes that were predominantly associated with estrogen signalling. While there are some remaining uncertainties regarding the precision with which reduced gene sets derived from EcoToxChips can predict BMDs in a regulatory context, this study suggests that tPODs derived from short-term exposures with non-protected life stages of fish show significant future promise to support chemical hazard assessment. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

1.02.T-05 Transcriptomic Profiles of a Respiratory Inhibitor and Growth Targeting Insecticide Reveal Links to Impaired Bone Mineralization and Lipid Homeostasis in Zebrafish Embryos

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Classical acute chemical hazard assessments focus on determining the concentration level at which a compound demonstrates adverse effects. However, this provides little to no mechanistic insights about the observed effects and is even less informative for sublethal and low concentration effects e.g. on the endocrine or reproductive system. Combining system biology methods (e.g. transcriptomics) with classical assays allows for the identification of molecular compound-specific fingerprints in a model system. To derive accurate adverse effect predictions from molecular fingerprints, mode of action (MoA) specific markers need to be identified. Therefore, we tested two insecticides with distinct MoA in the target organism in a modified version of the Fish Embryo Acute Toxicity test (OECD 236). Fenazaquin (FAZ) is a respiratory chain inhibitor targeting the mitochondrial complex I electron transporter, while pyriproxyfen (PPF) is a juvenile hormone mimic in insects and was recently shown to induce reproductive endocrine effects in zebrafish. Both compounds were tested at two different sublethal exposure conditions (low=LE and high=HE). At 96 hpf, total RNA was extracted, and subjected to full mRNA sequencing. Transcriptomic profiles were

screened for common sets of differentially expressed genes (DEGs) among both treatment conditions and then functionally linked to respective biological processes using overrepresentation analysis (ORA). Based on the selection of 127 potential marker genes, our analysis showed distinct gene clusters enriched between the two different MoA on the transcript level. On the functional level this pattern is conserved as both compounds interfere with distinct biological processes. PPF primarily affects steroid metabolic processes, estrogen response-related stimuli and lipid homeostasis. In contrast, FAZ affects the cell tissue organization and bio mineral tissue development, consequently affecting bone mineralization. We show that the integration of transcriptomics into a modified version of the acute zebrafish embryo toxicity test can be used to differentiate the molecular profiles of respiratory chain inhibitor FAZ from potentially endocrine active PPF, even at low exposure concentrations. We demonstrate that the combination of system biology methods with ecotoxicological test guidelines is an effective method to understand and distinguish underlying molecular mechanisms of adverse effects in non-target organisms.

1.02 Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future (Part II)

1.02.T-07 A Framework to Demonstrate the Applicability of New Approach Methodologies in Environmental Risk Assessment

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Current regulatory frameworks for assessing the environmental safety of chemicals are centered around in vivo data for standard representative species. This traditional approach is based on apical effects studies performed on whole organisms. However, there is a regulatory and ethical drive towards a paradigm shift incorporating new approach methodologies (NAMs) and a greater mechanistic understanding of toxicological responses across species. Mechanistic-based approaches allow the generation of points of departure (PoDs) (e.g., NOEC and/or LC50 and functional endpoints), to identify mechanisms of action and a variety of biological effects. However, there are still few examples of the application of these data in ERA. Here we propose a framework, exemplified with a case study by using Chlorpyrifos (CPS), for the use of NAMs in ERA to demonstrate the utility and the applicability of mechanistic-based information to complement and strengthen current ERA practices without the need for generating new animal data. The overall strategy involves the collection of effects data to provide a comprehensive set of PoDs including in vivo, in vitro, and in silico endpoints. Analysis of cross-species extrapolation approaches pointed that CPS molecular targets (ACHE/BCHE) and toxicity pathways are conserved throughout the animal kingdom. This information when coupled with bioactivity data from several sources confirmed the mechanism of action of CPS as ACHE Receptor Agonist, as well as invertebrates as the most sensitive taxa, specifically crustaceans. This case study demonstrates that the integration of traditional in vivo data and in vitro functional assays coupled with computational tools can build confidence in safety decision-making, validating our proposed framework for the use of NAMs in ERA. This framework provides confidence that invertebrates are the most sensitive taxa; sensitivity of most species where the target and pathways are conserved is either similar or less sensitive than crustaceans and highlights the potential use of a wealth of mechanistic data sources to inform safety decisions for data-poor (traditionally in vivo) chemicals as a surrogate. The results show this framework can be used as part of a weight of evidence approach to provide increased confidence in decisions making, and the insights will help gain a better mechanistic understanding of the potential expected toxicity effects.

1.02.T-08 Design and Optimization of a Serum-Free Culture Medium for the Rainbow Trout Cell Line RTgill-W1

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Efforts to replace fish for regulatory testing have resulted in the development of strategies and assay procedures based on permanent fish cell lines, especially of rainbow trout (*Oncorhynchus mykiss*). At the core of one such assay, capable to predict fish acute toxicity on chemical exposure, is the rainbow trout gill cell line (RTgill-W1). This assay has recently been approved by international standardization organisations, leading to the ISO standard 21115 and the OECD test guideline 249. However, while the assay as such is performed in a simple exposure medium without addition of animal components, RTgill-W1 cells are grown routinely in Leibovitz' L-15 medium (L-15) supplemented with 5-10% fetal bovine serum (FBS). FBS is a problematic supplement, both scientifically and ethically. We therefore have set out to develop a serum-free formulation, capable of supporting RTgill-W1 cell proliferation, by a systematic, bottom-up medium design. A high-throughput 96-well plate RTgill-W1 cell proliferation assay was developed and optimized to enable systematic screening of serum-free media components, individually and in mixture. Numerous supplements were tested. Some improved metabolic activity compared to cells in L-15, while others led to significant cell proliferation, comparable to cells cultured with 5% FBS. This was confirmed by direct whole-well cell counting after staining of cells DNA. The novel medium formulation enables RTgill-W1 cells to proliferate in the absence of serum. Fine-tuning of supplement concentrations, relying on rational experimental design for response surface methodology, will allow for a fully optimized medium design. Adaptation of RTgill-W1 clones to this new serum-free medium, including attachment, passaging and freeze/thawing are well underway – we may not be far from having the first fully defined culture medium for at least one type of fish cells.

1.02.T-09 Intrinsic Clearance Efficiency of the Three-Dimensional Rainbow Trout (*Oncorhynchus mykiss*) Hepatocyte Model When Assessing Three Different Fragrance Chemicals

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Hydrophobic chemicals which are not biotransformed have the potential to bioaccumulate in tissue and lipid reservoirs of aquatic organisms such as fish, causing both short and long-term biological effects. The assessment of a chemical's potential to bioaccumulate in fish requires determination of its physicochemical properties (e.g. log Kow) as a screening method or conventional animal (*in vivo*) test methods (OECD TG 305). Due to ethical and economical concerns, the development of alternative methods to measure *in vitro* biotransformation rates in primary hepatocytes (RT-HEP) and S9 fractions (RT-S9) from rainbow trout were established (OECD TG 319A/B) to improve existing *in silico* predictions. Due to the limited lifetime of RT-S9 and RT-HEP, these assays are not suitable to measure very slowly biotransformed chemicals. Three dimensional hepatic spheroids from rainbow trout (RT-SPH) with a longer assay duration has recently developed. The aim of this study was to determine the biotransformation rates of three different fragrance chemicals (log Kow 4.5-5.1) using RT-SPH, one slowly (Cashmeran, CASH), one moderately (Ambrofix, AMB) and one rapidly (Cyclohexyl salicylate, CS) biotransformed in the TG 319 A/B assays. The RT-SPH viability was measured during the full period of incubation (0-72 h) and decrease of the parent chemicals in active and heat-inactivated spheroids analysed by GC-MS. The *in vitro* intrinsic clearance rates ($CL_{IN\ VITRO,INT}$) were compared amongst RT-SPH, RT-S9 and RT-HEP. The RT-SPH were viable and a log-linear depletion was obtained during the whole period of incubation (up to 72 h) for all chemicals tested. Around 50% of the initial amount of CS, AMB and CASH was biotransformed between 4 and 48 h. Decrease in the heat-inactivated control was negligible (< 20%) for all three chemicals. The $CL_{IN\ VITRO,RT-SPH}$ rates of AMB and CS were 5- and 25- fold lower, compared to the RT-HEP $CL_{IN\ VITRO,INT}$. CASH, which is slowly biotransformed in both RT-S9 and RT-HEP, displayed a similar CL rate in the RT-SPH. However, extrapolation to the *in vivo* whole body biotransformation rate (K_B) displayed the RT-HEP and RT-S9 (0.05 and 0.06/d, respectively) underestimated the *in vivo* K_B (2.11/d) which was derived from the BCF by a factor of ca. 35-40, whereas RT-SPH did so by a factor of 5 (0.43/d). The RT-SPH are highly metabolically competent for at least 72 h and may better reflect the *in vivo* scenario when measuring compounds with slow CL rates.

1.02.T-10 Cytotoxicity and Modes of Toxic Action (MoA) of Ionic Liquids (ILs)

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ILs have gained a broad interest because of their tunable physicochemical properties, which permits a wide range of industrial applications. In parallel, the rising concerns of ILs entering the environment have prompted efforts to gain knowledge about the adverse effects. Despite of increasing knowledge on their toxic effects, the modes of toxic action have been studied only in a limited number of cases and no consensus has been reached yet. *In vitro* assays using reporter genes enable to identify MoA of chemicals and their implementation in high-throughput screening (HTS) culminates in the Tox21 database where more than 10,000 chemicals are tested in ~50 *in vitro* bioassays. By employing HTS and Tox21 database analysis, this study aims to unravel the MoA of ILs to tease out structure-activity relationships. We tested 45 ILs in two reporter gene cell lines: AREc32 assay for the detection of oxidative stress response and AhR-CALUX assay for the detection of aryl hydrocarbon receptor activation. Additionally, the reported cytotoxicity data from IPC-81 assay was used. Effects and cytotoxicity data were expressed as the concentrations triggering 10% effect (EC_{10}) and viability inhibition (IC_{10}) and used to derive toxic ratio (TR) with a baseline toxicity QSAR using liposome-water partition coefficient ($\log K_{lip/w}$) as a descriptor. Concentration-effect data for the 74 ILs were extracted for 10 assays from Comptox dashboard and processed to calculate the specificity ratio (SR). ILs did not activate any reporter genes but showed pronounced cytotoxicity. The cytotoxicity was mostly higher than the baseline QSAR prediction and large differences were observed between cell lines. Notably, elongation of the side chain results in higher cytotoxicity, implying the side chain length can be responsible for the change of MoA of ILs. Specific effects were found in aromatase inhibition and mitochondrial membrane potential (MMP) assays from Tox21 data analysis. These findings can be supported by the structural similarity between known aromatase inhibitors and ILs. Moreover, many ILs as well as aromatase inhibitors were found to induce mitochondrial toxicity and apoptosis. Taken together, we speculated that aromatase inhibition and/or mitochondrial toxicity can be potential MoA of ILs. This work suggested an approach to use reporter gene assays and Tox21 data enabling to narrow the future research focus for better understanding on the MoA of ILs.

1.02 Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future (Poster)

1.02.P-Th001 Understanding Biotransformation of the 'Novel' Organophosphate Ester, Bisphenol-A Bis(Diphenyl Phosphate) (BPADP): In Silico, In Vitro and Non-Target Analysis (NTA) Approaches

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Organophosphate esters (OPEs) have seen increased production and use as both flame retardants and plasticizers, often as replacements of the several banned brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs). A growing trend in the industry towards larger and more complex OPE molecular structures adds a new dimension to the biological stability and biotransformation of longer chained, higher molecular weight, 'novel' OPEs. However, little is known about the environmental fate, stability and bioaccumulation of 'novel' OPEs due to a lack of toxicokinetic, biotransformation and

metabolism data in exposed biota. The objective of this study is to characterize the biotransformation of bisphenol-A bis(diphenyl phosphate) (BPADP) for the first time using an *in vitro* Wistar-Han rat liver based microsomal assay. In conjunction with the *in vitro* assay approach, *in silico* modelling and Non-Target Analysis (NTA) are applied to address the hypothesis that metabolites of BPADP include bisphenol-A (BPA) and diphenyl phosphate (DHP). Rat liver S9, microsomal and *in vivo* rat metabolites were predicted *in silico* using the publicly available OECD Toolbox v4.4.1TM. *In vitro* assays were conducted in accordance with published assay methods. The metabolism kinetics of BPADP were investigated to determine the concentration of BPADP that is $\gg 2x K_m$, ensuring maximal rate of reaction (V_{max}) and zero order kinetics. Zero order kinetics has been achieved at a dosing concentration of 2 μ M for multiple OPEs, thus BPADP depletion and DHP formation were quantified during a 10 minute incubation at a concentration range between 0.5 μ M (356ppb) and 2.5 μ M (1780ppb). Time-dependent biotransformation assays were conducted following the same procedure, over a two-hour incubation. *In silico* modelling predicted DHP as both an *in vitro* and *in vivo* rat metabolite, with BPA predicted as the most probable *in vivo* rat metabolite. BPADP is shown to undergo *in vitro* microsomal metabolism with an average (n = 3) 44.6% BPADP depletion. BPA remains unconfirmed as an *in vitro* metabolite of BPADP, however DHP can be confirmed with a low but quantifiable average 1% recovery of DHP. NTA of *in vitro* samples via Liquid Chromatography coupled to a Q-Orbitrap-HRMS/MS are ongoing. Biotransformation was found to occur at a slower rate compared to legacy OPEs such as TPHP, which is likely attributed to the complex structure, high molecular weight and low solubility of BPADP.

1.02.P-Th002 Understanding the Drivers of Quantitative Structure-Activity Relationships' Predictability

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Alternative techniques to animal testing for chemical risk assessment are needed to face the ever-increasing number of new chemicals brought to the market. Quantitative Structure-Activity Relationships (QSARs), are one example of approaches used to infer on chemical toxicity without conducting animal tests. QSARs rely on different modelling techniques using everything from basic molecular properties, quantum mechanics to biological or environmental characteristics to estimate toxicity values for taxa or individual species based on a linear regression or machine learning algorithms. The predictive power of these QSARs is, however, not always tested on a fully independent dataset. Furthermore, it is not clear how the predictive power of QSARs can be improved. Here, we aim to investigate the predictive power of a diverse set of published QSARs for freshwater toxicity with an external dataset and to evaluate which QSAR characteristics mainly determined the differences in predictive power. First, a QSAR database was created gathering QSARs estimating (no-)effect concentrations of freshwater species. So far, 551 different QSARs from 113 studies estimating 17 different endpoints for 41 distinct species were gathered. Second, measured effect data for a large set of chemicals was gathered. Third, standard chemical properties of the chemicals for which measured effect data was available were estimated using the PaDEL and OPERA software. The QSARs gathered were then applied to the chemical dataset, while excluding estimates outside their applicability domain. Finally, the predictive power of each QSAR was estimated over all chemicals and species combinations if effect data was available for at least 10 different chemicals using the Nash Sutcliffe model efficiency coefficient (NSE). Only 49 QSARs could be fitted to the final set of chemicals with measured effect data (N = 2396) and only 34 yielded results for at least 10 chemicals. The NSE for these QSARs ranged from -66 to 0.89, with half of the QSARs having NSE above 0.2. More research is needed to understand this very large variability in QSAR performance and to explain why the predictive power of some QSARs is worse than the grand mean across effect data (NSE < 1). The next steps will address (i) the inclusion of additional QSARs in the database and (ii) the development of linear mixed effect models to understand which QSAR characteristics drive the predictive performance.

1.02.P-Th003 OECD Validation of the Rapid Androgen Disruption Activity Reporter (RADAR) Assay

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It is now widely accepted that a large number of environmental pollutants can interfere with the normal functioning of the androgen axis. Testing of androgen axis activity under REACH in whole organism models would require the use of huge numbers of adult or juvenile fish and mammals. We developed a transgenic medaka line capable of revealing the level of activity of the androgen axis by emission of green fluorescence. This stable transgenic line harbours a portion of the *spiggin1* gene promoter upstream of GFP coding sequence. The *spiggin1*-GFP line responds specifically to androgen axis signalling. It is capable of identifying pro-androgenic and anti-androgenic chemicals acting via a variety of modes of action (MoAs) such as androgen receptor agonists and antagonists, aromatase inhibitors, aromatase expression inducers or repressors, 5 α -reductase inhibitors and estrogens. The sensitivity of the line is similar to that of the 21-day androgenised female stickleback screen and uses eleutheroembryonic life stages, which are non-compliant with the EU definition of a laboratory animal. The RADAR assay is performed with twenty newly hatched transgenic eleutheroembryos of the *spiggin1-gfp* medaka line per exposure condition. The assay is performed in six-well plates for 72 h with media renewal every 24 h and can be automated, including the readout. The eleutheroembryos are exposed to test chemicals in the presence and absence of 3 μ g/L of 17 β -methyl testosterone, allowing quantification of the effects of chemicals acting via MoAs requiring the presence of an aromatisable androgen (e.g., aromatase inhibition, androgen receptor antagonism) as well as those more easily detected in the absence of an aromatisable androgen (e.g., androgen receptor agonists). **1. 2. 3.** During the OECD interlaboratory validation study, it was demonstrated that the RADAR

assay is transferable between laboratories. It can be read using a variety of manual or robotised fluorescence imaging systems and can be performed with either embryos shipped from a supplier or with embryos bred on site. Results of the validation study matched the expected results and a draft test guideline is currently in the final stages of evaluation by the OECD. The RADAR assay provides an ethical solution to reduce the number of adult fish used for endocrine testing.

1.02.P-Th004 Science Policy on 3Rs and NAMs - a Current Overview of Opportunities for Implementation in Upcoming Regulation Revisions

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Over the last decades ecotoxicological and toxicological research developed a wide range of alternative approaches to animal testing (or New Approach Methodologies (NAMs)) that are available or will soon be available for regulatory use. In parallel, the European regulatory landscape has created several opportunities for uptake of NAMs. However, uptake of NAMs by regulators is still very limited to this day. With the upcoming revisions of REACH, CLP and the Cosmetics regulation in 2022 as well as the implementation of the EU Chemical Strategy for Sustainability a series of opportunities for inclusion of NAMs is happening. Current initiatives in regulatory policy and science in the EU were reviewed and analysed for importance for the SETAC community. On the political level, the European parliament recently adopted a resolution calling for an action plan to replace animal testing in education, science and regulatory testing. The Resolution created a political momentum that can be used in view of the upcoming regulatory revisions. On the level of EU agencies, EFSA and ECHA both are launching different initiatives with the ambition to accelerate the uptake of NAMs in regulatory science in the EU. In regards to new research initiatives, there are a number of projects, clusters and partnerships that work at the science-policy interface on the topic of NAMs. The European Partnership for the Assessment of Risks from Chemicals (PARC) will develop a Next Generation Risk Assessment road-map. PARC constitutes a unique opportunity to bring together the European communities of regulators, researchers and industry that are involved in risk assessment of chemicals. The Animal-free Safety assessment of chemicals: Project cluster for Implementation of novel Strategies (ASPIS) launched in 2021. The cluster closely cooperates with European institutions such as the EC DG JRC to enable integration of research findings into regulatory legislation such as REACH updates testing requirement. The European Partnership for Alternative Approaches to Animal Testing (EPAA) supports projects that contribute to the acceptance and implementation of NAMs. A recent EPAA workshop presented the successful application of NAMs for a variety of endpoints to particularly tackle worker exposure under REACH. This overview analysis found projects, activities and upcoming regulatory revisions that can be used to develop a blueprint for future activities of SETAC members in the area of NAM research.

1.02.P-Th005 A Semiautomated High Content Workflow for Comparative Assessment of Chemical Effects in *Danio rerio* Embryos

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The major goal of chemical risk assessment is to identify the potential adverse health or environmental effect of chemicals. In this context, animal testing played a central role in predicting the effect of hazardous chemicals on human health. Although it has been widely used to predict adverse effect in humans, animal testing using adult animals has started to raise many ethical and economic concerns. The EU project PrecisionTox aims at identifying hazards of chemicals using a suite of non-mammalian alternative model organism and in vitro tests, including the zebrafish embryo test. Embryos of the zebrafish (*Danio rerio*) represent a valid alternative to standard animal testing and align with the 3Rs concept. Therefore, the main goal of the present study is to apply a previously developed semi-automated high content workflow to assess the adverse effect of 250 chemicals using automated imaging and behavior tracking for the assessment of developmental (neuro)toxicity. Zebrafish eggs were exposed in 96-well plates for 96 hours to chemicals covering a wide spectrum of mode of actions. Embryotoxicity was assessed at 24 and 96 hpf by monitoring mortality, motor behavior and morphological changes. Motor behavior was assessed using a video tracking system under different light conditions and morphology was assessed at 96 hpf. Preliminary results showed specific effect patterns in zebrafish embryos. Cadmium chloride caused strong teratogenic effects and 15 different type of malformations. Ethoprophos led to a decrease in all types of analyzed motor behavior without causing mortality or altered phenotypes. Sodium arsenite caused a reduced activity on the STC and PMR at concentrations higher than the mortality benchmark dose. It showed to the highest number of malformations if compared to the other four chemicals. Unexpectedly, pirinixic acid a general decrease in the motor behavior and 7 type of malformations. Finally, dimethyl sulfoxide showed a weak effect on nearly all endpoints. Our preliminary results support that a semi-automated assessment of chemicals *Danio rerio* is possible and that distinct effect patterns can be obtained. Effect levels will be compared with predicted unspecific effect concentration, internal concentrations and used to classify chemicals by their toxicologically relevant mode of action, e.g. for application of read across of chemicals for human toxicity.

1.02.P-Th006 Transcriptomic Points-Of-Departure (tPODs) at Early LIFE Stages of Phylogenetically Distant Fishes Exposed to 17 α -Ethinylestradiol and Fluoxetine

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The development of new approach methods (NAMs) that aim to reduce the use of live animals in toxicity testing is urgently

needed to meet current regulatory mandates to assess the toxicological risks of the vast and rapidly increasing number of chemicals used by society. However, many NAMs including current *in vitro* and *in silico* systems are limited in accurately estimating apical toxicity thresholds as they do not represent the complex interactions occurring within an organism after a toxic insult. This study aimed to use a short-term embryonic exposure assay with three phylogenetically distant ray-finned fishes to derive transcriptomic points-of-departure (tPODs) and to compare these with apical PODs. Two emerging contaminants of concern, 17 β -ethinylestradiol (EE2) and fluoxetine (FLX) were used as test chemicals. Embryos of white sturgeon (WS), rainbow trout (RBT), and fathead minnow (FHM) were exposed to graded concentrations of EE2 and FLX until four days post-hatch and subjected to mRNA sequencing. Transcriptomic benchmark dose (BMD) analyses yielded tPOD estimates that closely approximated apical PODs found in the literature. tPOD estimates for the median of the 20 most sensitive gene BMDs (omicBMD₂₀) were the most protective estimates across species and across chemicals, while annotation-based tPOD estimates derived from pathway enrichment analyses (pathBMD) appeared to be less sensitive. tPODs from RBT were the most protective of chronic apical effects for both EE2 and FLX, while FHM was the least sensitive species. This study highlighted that, despite some remaining uncertainties, tPODs derived from short-term non-protected embryo-larval/alevin exposures were protective of chronic apical PODs, and therefore, show significant promise as a NAM to support chemical hazard assessment and regulatory decision making.

1.02.P-Th007 Development and Characterization of a Double-Crested Cormorant Hepatic Cell Line for Chemical Screening

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There are currently no available cell lines for the ecologically relevant colonial waterbird species, the double-crested cormorant (DCCO). DCCOs are high trophic level aquatic birds that are used for routine contaminant monitoring programs in the Laurentian Great Lakes and marine coasts of Canada. Developing a DCCO cell line for *in vitro* toxicological screening will ideally provide improved understanding of the effects of environmental chemicals given the large differences in sensitivity between laboratory and wild avian species. In this study, an immortalized DCCO hepatic cell line, DCH22, was established from the liver of a day 22 embryo as a potential alternative to primary double-crested cormorant embryonic hepatocytes (DCEH) for chemical screening. DCH22 cells were cultured for over a year and had hepatocyte-like morphology. Exposure to PCB-126 induced CYP1A activity in DCH22 cells cultured as 2D monolayers and 3D spheroids. Induction of CYP3A activity was observed following exposure to carbamazepine, dexamethasone and metyrapone in 3D spheroids. Preliminary results suggest that DCH22 cells have CYP1A and CYP3A activity supporting their potential use as an alternative to primary DCEH for chemical screening. This cell line will be used to screen benzotriazole UV stabilizers (BZT-UVs), among other priority chemicals, in future studies.

1.02.P-Th008 Cytotoxicity of Sea-Dumped Chemical Munitions in Rainbow Trout Gill (RTgill-W1) and Human Colon Adenocarcinoma (Caco2) Cells

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Following the First and Second World Wars colossal amounts of munition, including conventional explosives and chemical warfare agents, were sea-dumped in coastal and off-shore sites all over the globe. As a consequence of decades of exposure to seawater, munition shells are expected to be highly corroded thus allowing the release of toxic chemicals to the environment. Despite the growing environmental concern, the toxic potential of dumped munition and their reaction products is largely unknown. In this study, the cytotoxicity of thiodiglycol, 1,4-oxathiane and 1,4-dithiane, three metabolites of the chemical warfare agent sulphur mustard frequently detected in environmental samples from dumpsites, was assessed in rainbow trout gill (RTgill-W1) and human colon adenocarcinoma (Caco2) cells following 24 hours and 24 and 48 hours exposure, respectively. For that, a combination of three viability assays was used following the test guideline 249 from OECD. The obtained results for RTgill-W1 allowed the estimation of the LOECs of 50 mg/L and 100 mg/L for 1,4-dithiane and 1,4-oxathiane, respectively, which correspond to the 96 hours LOEC in fish, while a NOEC of 100 mg/L was estimated for thiodiglycol. Similarly, for Caco2, 1,4-dithiane also proved to be the most toxic of the tested chemicals with the LOECs of 6.25 mg/L and 100 mg/L, following 24 and 48 hours exposure. Furthermore, LOECs of 100 mg/L were estimated for 1,4-oxathiane and thiodiglycol following both exposure periods. This first insight on the cytotoxic potential of three relevant sulphur mustard metabolite products reveals that 1,4-dithiane is the most toxic of the tested chemicals, even though all estimated LOECs are well above the concentrations detected in environmental samples, which normally range $\mu\text{g/L}$.

1.02.P-Th009 EcoToxChip Test System: A Toxicogenomic New Approach Method (NAM) for Chemical Prioritization and Environmental Management

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In response to regulatory and stakeholder calls for New Approach Methods (NAMs), since 2016 our team has set forth to develop, test, validate, and commercialize toxicogenomic solutions (i.e., EcoToxChips, and a data evaluation tool EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. EcoToxChips have been developed for common laboratory model species representing the most important vertebrate groups in ecological risk assessment (fish-fathead minnow; bird-Japanese quail; amphibian-*Xenopus laevis*), and EcoToxChips for key native species are expected in early 2022 (rainbow trout, Northern leopard frog, double-crested cormorant). EcoToxChips are now

being tested according to a validation plan that has been reviewed by 5 offices in Environment and Climate Change Canada (notably, the Biological Assessment and Standardization Division) along with key project partners (US EPA, US Army Corps of Engineers, Shell, USGS, Eurofins) and social sciences researchers in the team. To date, more than 1,170 EcoToxChips have been analyzed and in this presentation we will summarize key findings from our case studies. In brief, case studies to date consist of 28 distinct chemicals being tested as well as 4 complex environmental samples. Two of the chemicals are being tested in a dose-response manner in three independent laboratories. In addition, 4 of the chemicals are tested in multiple species (species read-across), and 4 chemicals are being tested both in vivo and in vitro. For the majority of test chemicals, dose-response (i.e., to derive transcriptomics points of departures) and RNA-seq data (i.e., to evaluate cross-platform comparisons) are available. Exposure studies are complete for 22/28 chemicals and all 4 complex environmental samples. Notably, 10 of the 28 chemicals are purposefully included for regulatory interests. The interpretation of EcoToxChip results is facilitated through a user-friendly and intuitive cloud-based tool (EcoToxXplorer.ca) that continues to evolve based on feedback. EcoToxXplorer now integrates with information from the AOPwiki and includes our custom EcoToxModule gene sets designed for high-level interpretation of toxicogenomics data. Taken together, these diverse studies are demonstrating how the EcoToxChip Test System can serve as a toxicogenomic New Approach Method that is accessible, standardized and user-validated. Importantly, many partners have regulatory interests, and so the case studies are helping us to purposefully design the EcoToxChip Test System to be one that can be used quantitatively as a decision support tool in regulatory applications including chemical prioritization and guideline development. This study was conducted as a part of a large-scale Genome Canada-funded project (EcoToxChip project - www.ecotoxchip.ca).

1.02.P-Th010 Phytochemical Profile of the Extracts of *Bidens Pilosa* and Its Correlation With Antioxidant ACTivity, Cell Cytotoxicity, and Larvicidal ACTivity

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Bidens pilosa L. has traditionally been used as a flavorant in foods, in the treatment of diseases, and in agriculture as a biopesticide, with no reports of obvious adverse effects. The aims of this study were: i) to determine the cytotoxic effects of 70% ethanol and water extracts of *B. pilosa* on cell cultures using real-time cell analysis (RTCA); ii) the antioxidant activities; iii) larvicidal activities using the SYBR Green I-based fluorescence assay; and iv) to determine the phytochemical profiles using liquid chromatography coupled to a quadrupole-time-of-flight (LC-ESI-Q-TOF-MS) mass spectrometer (Waters Synapt G2). The quantitative analysis of the phenolic compounds in the crude extracts was determined using chemical analysis. The extracts show dose-dependent kinetics. However, only the ethanol extracts exhibited cytotoxic activity on the human liver, HepG2 (cancerous), and African monkey kidney, Vero (non-cancerous) cell lines. At 20 µg/mL, the water extract inhibited *Plasmodium falciparum* by 79.70% and was more potent than the ethanol extracts (inhibition; 30.43%), contrary to the cytotoxic effects shown in RTCA. The ethanol extracts expressed pronounced antioxidant activity and cytotoxicity, probably due to the presence of a higher concentration of flavonols, flavanones, and other polyphenols such as quercetin and kaempferols, in the ethanol extract as compared to the water extract. This study shows that ethanol extract of *B. pilosa* is more potent and could yield better results when used traditionally or for other purposes.

1.02.P-Th011 Comparison of In Vitro to In Vivo Data Generated in Bioaccumulation Studies

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Fish bioconcentration or biomagnification studies are carried out to determine whether substances have the potential to bioaccumulate and have potential to bio-magnify throughout different trophic levels. There are increasing requirements to conduct these studies on a broad variety of industrial chemicals, pharmaceuticals and crop protection products across the globe, resulting in the use of significant numbers of fish. The preferred method of testing requires three doses of chemical (including a control) with up to 100 fish per dose. However, following the revision to the OECD Test Guideline (OECD 305: 2012) there is now the option to test on one concentration and the control only, providing there is scientific justification. This has the potential to decrease the numbers of fish used in these tests by one third (100 fish per study). There are opportunities to reduce animals further by following new in vitro alternatives such as the OECD 319 study (Determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes). The liver intrinsic clearance is used directly as an input to physiologically based toxicokinetic (PBTK) models for fish bioaccumulation. Alternatively, this value may be extrapolated to a whole-body (in vivo) biotransformation rate constant using an appropriate in vitro to in vivo extrapolation (IVIVE) model. The in vivo biotransformation rate can be included into in silico models for prediction of bioconcentration factors (BCF). Although novel validated methods exist to conduct in vitro fish bioaccumulation studies, regulators are not yet confident to accept these studies in isolation without supporting in vivo data. This is because they have not seen enough studies of this type submitted and do not have confidence in the direct in vitro/in vivo extrapolation. Direct comparison of data generated both in vivo and in vitro on the same chemicals is therefore needed to drive regulatory acceptance. Here we present results we have obtained in our laboratories comparing predicted BCF values obtained in vitro to those conducted in vivo in an OECD 305 setting on the same test materials.

1.02.P-Th012 Do We Really Need Acute Fish Toxicity Testing in Pesticide Risk Assessment?

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The current risk assessment (RA) of plant protection products (PPPs) for the aquatic environment is based on the evaluation of the toxicity to various taxonomic groups including extensive vertebrate studies with fish, both acute and chronic. According to the aquatic guidance document, the specific protection goal (SPG) for aquatic vertebrates “aims at protection at the individual level, so that mortality and suffering due to acute toxicity is avoided”. Hence, one of the tier 1 requirements for the authorization of PPPs is the performance of acute fish studies, which is laid down in the Commission Regulation (EU) No 283/2013 for active substances and the Commission Regulation (EU) No 284/2013 for PPPs. However, the Directive 2010/63/EU on the protection of animals used for scientific purposes and the Regulation (EC) No 1107/2009 clearly require the minimization of vertebrate testing. Considering the 3Rs principle (replacement, reduction, refinement), the current approach of testing fish toxicity in both acute and chronic studies needs to be critically reviewed. Therefore, it was investigated whether acute fish toxicity studies are drivers for the overall aquatic RA of pesticides and thus must be deemed necessary to ensure a safe use of PPPs or whether the acute fish toxicity can be covered by either chronic fish studies or other taxonomic groups. For this purpose, we compared the toxicity of 47 active substances comprising 14 fungicides, 21 herbicides and 12 insecticides to fish, invertebrates, sediment dwellers, algae and macrophytes using EU agreed endpoints. Preliminary results demonstrate that the acute toxicity to fish is usually well covered either by chronic fish studies or by other taxonomic groups. The evaluation shows, that the implementation of acute fish toxicity studies is not necessarily required to address the SPG for the aquatic environment. Indeed, it is indicated that there is huge potential to minimize vertebrate testing and thus implement the 3Rs principle in reducing the number of fish to be tested. The use of alternative methods such as the Fish Embryo Acute Toxicity (FET) Test (OECD Test No. 236) or the newly developed Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay (OECD Test No. 249) might be two promising methods that could replace acute fish toxicity studies in a comprehensive non-vertebrate testing strategy in future without reducing the protectiveness for the aquatic environment and its species.

1.02.P-Th013 Benzalkonium Chloride-Induced Cytotoxicity in Human Cell Lines

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Benzalkonium chloride (BAC) has been used worldwide safely for decades, is a safe and effective product with sterilization effect. However, when inhale BAC, it is known to show extremely high toxicity. Recently, the use of hand sanitizers and disinfectants is increasing due to the COVID-19 pandemic. Therefore, safety evaluation is necessary because it is highly likely to cause toxicity due to directly exposure to the lung and skin. In this study, we evaluated in vitro toxicity of BAC in human lung fibroblast cells and human keratinocyte cells. We showed that BAC induced apoptosis and increased mitochondria dysfunctions including reactive oxygen species (ROS) production and regulation of the mitochondrial permeability transition pore (mPTP). Mitochondrial STAT3 is known to relate to ROS production and mPTP and it was reduced by BAC and hand sanitizer containing BAC. In addition, we confirmed that knockdown of STAT3 reduced the cell viability and reactive oxygen species increased compared to the control group. In conclusion, our results suggest a method to evaluate toxic effects of BAC via mitochondrial STAT3 protein.

1.02.P-Th014 Application of a Biotechnological Metabolization System to Improve the Forecast Power of the Fish Embryo Acute Toxicity Test

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The Fish Embryo Acute Toxicity (FET) Test, according to OECD 236, is an early-life-stage test to evaluate lethal and sublethal effects of chemicals/chemical mixtures on the organismic level of the zebrafish (*Danio rerio*) during the first days of development post fertilization. It was developed and standardized as a substitute for the acute fish test according to OECD 203, in which effects of the test substance/mixture on adult fish are assessed. The use of larvae is considered as a replacement of the more sentient adult animals according to the 3 R's principle (reducement, refinement and replacement of animal experiments), first invented by Russell and Burch in 1959. However, *Danio rerio* larvae have only limited metabolic capacity during the development until 96 hours post fertilization compared to adult fish. This can result in an under- or overestimation of the toxicity of environmental samples or chemical substances. Therefore, the FET is poorly established/accepted in the regulatory context as an alternative to the acute fish test. Thus, this project is intended to investigate whether the FET could be further improved by a preceding metabolism of the sample and thus strengthen its forecast power for regulatory risk assessment. For pre-metabolization, a biotechnological, animal component-free S9 and a conventional S9-fraction (liver homogenate) are used and compared. Both are used to enable the detection of the metabolite's effects in the FET. The effects observed during the test using pre-metabolized substances will be compared to the effects resulting from the non-metabolized parent compounds. Through biotransformation, stronger or weaker effects could then be monitored in the FET, which may better reflect the results from the acute fish test with adult fish with full metabolic capacity. For this study, five model substances are used for which bioactivation is expected to increase their toxicity. It is assumed that both testing approaches result in different concentration-response curves, where (possibly different) (sub-)lethal effects may occur at lower concentrations with pre-metabolized substances compared to the approach using parent compounds. The study aims to develop an applicable method to make the FET results more comparable to the acute fish toxicity data and therefore even more relevant for risk assessment.

1.02.P-Th015 Hepatic 3D Spheroids: The Next Generation of Testing in Toxicology (SPHERTOX)

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Alternatives to animal testing have long been sought by implementing the 3Rs (refinement, reduction and replacement), to more efficiently assess the safety of chemicals, endorse ethical considerations and reduce costs of experimental studies. Regulatory legislations have in recent years required more chemicals to be assessed for hazard, increasing the need for toxicity and bioconcentration (BC) testing. Thus, efforts to implement (non-animal) alternative bioassays, with key focus on fish, has intensified. Recent advances within *in vitro* fish acute toxicity and BC assessment have resulted in standardized tests with short exposure times. There is currently no satisfying *in vitro* model accommodating chronic toxicity testing and/or BC assessment of chemicals with low biotransformation rate. The primary objective of the SPHERTOX project is therefore to assess the performance of hepatic 3D spheroids from rainbow trout as an alternative chronic toxicity- and bioconcentration system, alone or in co-culture with other cell types, as an alternative and advanced *in vitro* model with increased comparability towards *in vivo* test systems. To fulfil this objective, SPHERTOX will generate *in silico* and experimental data to develop and evaluate novel and alternative ecotoxicological test methods for fish, with the following main aims: Characterisation of the spheroid model's morphological and physiological properties; Establishment of 3D spheroids as a chronic low-dose and repeated exposure *in vitro* model; Application of spheroids to measure low biotransformation rates of chemicals in bioconcentration assessment; Generation of a multi-compartment co-culturing system using hepatic spheroids and primary gill cells for acute and chronic toxicity testing; Improvement of *in vitro* to *in vivo* extrapolations by using 3D spheroids alone and/or in co-culture; Development and implementation of a causal inference model to identify mechanistic interactions and confounding factors within *in vitro* and *in vivo* toxicity testing. This project seeks to provide both basic and applied knowledge by generating theoretical and experimental data to develop and evaluate the next-generation ecotoxicological methodologies for fish using cutting-edge analytical technologies. The SPHERTOX project is funded by the Norwegian Research council (Project: 324794).

1.02.P-Th016 Modulation of Transient Reporter Vectors of the Xenobiotic Metabolism Pathway in Zebrafish Hepatocytes

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Alternative *in vitro* test systems are in high demand within toxicology since the Tox21 paradigm shift in animal testing. Especially within ecotoxicology, coverage of aquatic species-specific assays is relatively scarce. Under these circumstances, transient reporter gene assays could be a quick, economical, and reliable solution. At least as a bridging technology. Nevertheless, users should be aware of inherent technological pitfalls regarding reporter gene assays, such as artefacts induced by reporter vector geometry. Here, we outline the development of an AhR-responsive transient reporter-gene assay in a permanent zebrafish hepatocytes cell line (ZFL), using both synthetic (multi-species consensus sequence) and endogenous, genomic (zebrafish) gene regulatory elements. Further, we disclose how viral, constitutive promoters (CMV, SV40, TK) located on the reporter-gene assay cassettes induce squelching of the primary signal. We designed a novel normalization vector, which bears an endogenous zebrafish-derived genomic promoter (zfEF1aPro) as a countermeasure. Our report depicts how the latter rescues the squelching-delimited system and gives insights into the modulation of transient reporter systems under xenobiotic stress. Lastly, we uncover a multi-level biological organization inhibitory mechanism that might mask effects ("maisonette squelching"). It appears that the ubiquitously used ligand BNF promiscuously activates multiple toxicity pathways of the xenobiotic metabolism and cellular stress response in an orchestral manner, presumably leading to a concentration-related inhibition of the AhR/ARNT/XRE-toxicity pathway and non-monotonous concentration-response curves. Conclusively, we prove transient, fish-derived reporter gene assays to be a potent tool in assessing molecular initiating events. However, we want to point out that such assays need careful development and increased quality control to avoid common technological shortcomings.

1.02.P-Th017 Combined In Vitro Exposure of Polystyrene Nanoparticles and North Sea Marine Oil: Assessing Sensitive Endpoints of Toxicity

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Over the last decade, there has been increased interest in the impact of plastic pollution on the aquatic ecosystem. Micro and nano plastics generated as a result of plastic degradation and the risks posed to aquatic life is of great concern. However, little attention has been given to interactions of these particles with other environment pollutants found in the aquatic environment. This study aims to gain knowledge on the possible toxic effects associated with single and combined exposure of Polystyrene nanoparticles (PSNPs) and water accommodated fractions (WAF) of North Sea marine oil (containing polyaromatic hydrocarbons (PAHs)) on fish *in vitro* models and explore more sensitive *in vitro* assays for the assessment of nanoparticle toxicity. Rainbow trout (*Oncorhynchus mykiss*) cell lines were exposed to a range of concentrations (0.1-100 µg/mL) of PSNPs (25, 100 and 2000nm) alone or in combination with North Sea oil WAFs. Endpoints measured included cytotoxicity using Alamar Blue and CFDA-AM probes, cytochrome P450 1A (CYP1A) activation (EROD activity) and generation of reactive oxygen species (ROS). Exposure to PSNPs alone demonstrated significant changes in cellular metabolic activity, membrane stability and generation of ROS in rainbow trout gill and liver cells. Changes observed were particle size and concentration dependent, little to no changes were observed with the 2000nm, viable cell counts ranged from 60-80% with 100nm and 30-45% in 25nm, suggesting increased toxicity with reduction in particle size. An increasing trend of ROS generation was observed in 25 and 100nm PSNPs exposure only as well as with WAF alone and trends in EROD activity was observed to be induced by WAF only. Pilot studies investigating the combined exposure of similar oil: water fractions and PSNPs demonstrated reduced cytotoxicity by 25-40% at select concentrations in rainbow trout gill and liver cells. In addition to *in vitro* toxicity studies, more sensitive assays including

gene expression analysis for markers of sublethal toxicity will be explored, effects on generation of intracellular ROS, CYP1A induction potential in rainbow trout cells as well as toxicity and behavioural effects in Zebrafish larvae will be conducted to gain an overall picture of the risk associated with these interactions.

1.02.P-Th018 Omics- Based Identification of MOLECULAR Effects of Fungicidal Active Substances in Zebrafish Embryo
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Active substances of fungicides introduced to the ecosystem in a targeted manner can exert adverse effects on non-target organisms, in particular populating soil and the aquatic ecosystem. Currently, the regulatory authorities prescribe a number of studies for the assessment of environmental side effects, including the analysis of bioaccumulation, environmental fate and toxicity in different model organisms such as earthworm, algae, water flea or fish. However, these studies do not cover substance-induced effects at the molecular level. The application of recent OMICS-technologies allows for a sensitive and global identification of molecular changes at the level of RNA (transcriptomics) and proteins (proteomics). This work aims at the identification of molecular changes induced by a number of FRAC (Fungicide Resistance Action Committee)-classified fungicidal active substances in zebrafish embryo in order to predict the adverse environmental effects and global gene expression changes induced by these substances. Zebrafish embryos were exposed to sub-lethal concentrations of fungicidal active substances for 96 hrs followed by simultaneous RNA and protein extraction from respective samples in order to analyse further effects induced by those substances in transcriptomic and proteomic profiles by applying RNA-Seq (transcriptome) and HPLC-Mass-Spec (proteome) analyses. The resulting molecular fingerprints will provide the basis for a mode-of-action (MoA)-specific screening of active substance precursors under development and monitoring of environmental samples in order to assess the environmental load of fungicide active substances. Deep knowledge about molecular changes induced by active substances with different (MoA) and the linkage with phenotypic and population effects will significantly facilitate the classification of active substances related to their environmental side effects.

1.02.P-Th019 The Onset of Active Gill Breathing in Zebrafish (*Danio rerio*) Larvae Correlates With an Increase in the Sensitivity to Neurotoxic Pesticides

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A major concern about the applicability of the fish embryo toxicity test (FET) as an alternative for the fish acute toxicity test (AFT) is based on the observation of low sensitivities to neurotoxic substances. Given that oxygen uptake in early developmental stages of fish embryos primarily occurs *via* diffusion across the skin, substances which impede or prevent active ventilation of the gills might lead to premature death in adult fish may not be effective in skin-breathing embryos. To investigate if this respiratory failure syndrome (RFS) plays a role for the higher sensitivity of adults, a new acute toxicity test using postembryonic, early gill-breathing life-stages of zebrafish was conducted with chlorpyrifos (CPS), permethrin (PM), lindane (?-HCH), aldicarb (ALD), ziram (ZDMC) and aniline (AN) as test substances. Additionally, a comparative study into oxygen consumption (MO_2), breathing frequency (f_v) and amplitude (f_{amp}), as well as swim bladder deflation as possible side-effects of the RFS was performed with 4 d old skin-breathing and 12 d old gill-breathing zebrafish. Acute toxicity tests revealed that postembryonic larvae represented the most sensitive life-stage for all test substances except PM, if compared to both FET and AFT. In 12 d old larvae, MO_2 , f_v and partly f_{amp} were significantly increased in all treatments, whereas pesticide exposure 4 d old embryos only caused minor effects. Whereas ZDMC and AN showed no significant effect on swim bladder deflation, CPS-, ?-HCH- and PM-exposed fish showed significantly deflated swim bladders mostly in 12 d old larvae, but, in case of ALD, a significant hyperinflation in 4 d old embryos. Swim bladder deflation in 12 d old larvae indicates a potential disruption of gas secretion homeostasis by systemic effects of the respiratory failure syndrome, whereas in 4 d old embryos choline-mediated hyperinflation of the swim bladder dominates over other effects. The induction of the respiratory failure syndrome during the onset of active gill respiration by neurotoxic pesticides apparently correlates well with differential sensitivities of skin-breathing embryos and gill-breathing later life-stages of fish. However, regarding acute lethality, this study also provides striking evidence that conventional toxicity testing with adult fish may also fail to give ecologically relevant predictions of acute toxicity, since adults do not necessarily represent the most sensitive life-stage.

1.02.P-Th020 SNAPFISH - Searching for Refined In Vitro Approaches to Predict Bioconcentration of Chemicals in Fish

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The ecotoxicological assessment of chemicals requires information about their bioaccumulation potential in organisms. This is particularly important for hydrophobic organic chemicals due to their high sorption and potential for bioaccumulation. Steady-state BCFs in fish reflect the net result of the various chemical uptake versus loss processes and can be determined directly (e.g., using OECD 305). However, in the context of the 3R-targets for the use of animals in research, alternative approaches to derive BCFs based on *in vitro* and/or *in silico* approaches are required. The main aim of the present study is to obtain *in vitro* metabolic rates from assays based on rainbow trout liver S9 fractions (RT-S9) for a set of reference chemicals, namely pyrene, deltamethrin,

4-*n*-nonylphenol, and methoxychlor. Secondly, the rates determined from these S9 *in vitro* assays shall be compared with several perfused liver experiments using the same strain of fish as for the S9 assays. This comparison provides an intermediate step for testing the *in vitro* – *in vivo* extrapolation (IVIVE) process that considers sorption processes and the different amounts of metabolically active components *in vitro* and *in vivo*. Furthermore, we will report on the application of partitioning-based dosing methods in the S9 *in vitro* assays for a more robust metabolic rate measurements of selected highly hydrophobic reference compounds at defined dissolved concentrations. These make use of albumin as the passive dosing phase to provide fast and reliable release kinetics in the assays. Abiotic partitioning experiments for the albumin and RT-S9 fractions have also been performed for the reference compounds to provide corresponding distribution coefficients. These measured data provide the free fractions of the reference compounds in the assay medium which is needed as part of the IVIVE. Both, the development of partitioning based dosing methods for S9 *in vitro* assays as well as the comparison of S9 *in vitro* data with perfused liver experiments shall finally lead to a refinement of the IVIVE model, which would in turn provide more reliable BCF estimates and thus better predictability of the environmental risk of hydrophobic chemicals.

1.02.P-Th021 A New Network of In Silico Models - LIFE CONCERT REACH Project

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This poster provides an update on the LIFE CONCERT REACH project, which started in 2018 and aims to establish a new, freely accessible, network of in silico models, including both (Q)SAR and automated read-across tools. The EU-funded project will be finalized in 2022 and involves scientific, regulatory as well as industry partners. The network of in silico models should be achieved by linking existing and well-known platforms (such as VEGA platform, the Danish QSAR Database, OCHEM and AMBIT), as well as developing and integrating new models, potentially covering all properties required within the framework of Regulation EC (No) 1907/2006 (REACH). The new network will comprise statistical and knowledge-based (Q)SARs as well as automated read-across tools. The goal of the project is to offer more than 300 freely available in silico models. An interface with the OECD QSAR Toolbox is also envisaged. Furthermore, compliance with current regulatory requirements with regard to reporting (i.e. QMRF and QPRF generation) is also an intended target. Here we present some of the new models, which have been implemented in the last VEGA platform version and are now publicly available. Furthermore, we will give an overall update on goals achieved within the LIFE CONCERT REACH project.

1.02.P-Th022 Sequential Exposure of Zebrafish Embryos to Two Differently Acting Insecticides - a CASE Study With Chlorpyrifos and Abamectin

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Neuroactive chemicals such as organophosphate and avermectin pesticides are frequently detected in the environment. Singly or in a mixture, they could provoke neurotoxic effects and neurological diseases to organisms. Understanding the effects of pesticide mixtures including substances with different mode of action would help in risk mitigation. In order to assess mixtures of neurotoxic compounds, we have used the zebrafish embryo model. Zebrafish embryos (0-120 hpf) are considered as an alternative to animal testing and are increasingly used for hazard assessment. In addition to standard OECD 236 testing with lethal and sublethal effects, recent efforts to expand effect parameters included the assessment of behavior or movement changes in fish embryos as a potential indicator of neuroactivity, e.g. the spontaneous tail coiling (STC). Here, analysis of changes in the STC was used to study the pesticides chlorpyrifos, chlorpyrifos-oxon and/or abamectin. The effect of co- and sequential exposures was analysed with STC and by measurement of the acetylcholinesterase (AChE) inhibition. Results show that both chlorpyrifos and its oxon metabolite induced hyperactivity in the STC test. Chlorpyrifos is a known inhibitor of acetylcholinesterase, but its activity is mediated via the active metabolite, chlorpyrifos-oxon. Coincidentally, only chlorpyrifos-oxon inhibited AChE while chlorpyrifos did not. Further, abamectin which acts by activating Gamma aminobutyric acid receptors (GABA_R) in invertebrates blocked the chlorpyrifos induced hyperactivity but not that of chlorpyrifos-oxon. These results point to a potential GABA_R-related or rather GABA_R analogue inhibition or cross reaction for the parent compound, chlorpyrifos, rather than the AChE inhibiting mechanism.

1.02.P-Th023 Application of In Vitro Fish Cell Line Assays to Predict the Mixture Toxicity of Plant Protection Products

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In the context of plant protection products registration, mixture toxicity is one of the most controversial topics in regulatory ecotoxicology. In order to estimate if mixture effects are either additive, antagonistic or synergistic, a comparison of the predicted mixture toxicity (based on single substance toxicity data) versus the experimental mixture endpoints is performed. The same applies for the mixture assessment of plant protection products in the European Union, where the calculated mixture toxicity is compared with the measured toxicity of the formulated product. Formulated products, however, do not only contain active substances but also comprise co-formulants, which are completely ignored in this comparison. Perhaps, the results are therefore inaccurate and could lead to misinterpretations of the underlying mixture toxicity effects. A much more accurate approach could be the concept of “isobolograms”. Isobolograms determine if a mixture shows additive, synergistic or antagonistic effects by plotting the concentrations of two chemicals against each other and representing the amount of the toxicants in the mixture which produces a given biological response. This approach, however, requires a large number of assays on vertebrate species and is

obviously in contrast to the effort that is made to reduce the amount of regulatory vertebrate testing. To explore the mixture toxicity of plant protection products, the present study utilized a rainbow trout gill cell line assay (RTgill-W1, ISO guideline 21115, which served as the basis for the recently adopted OECD test guideline 249). Recent studies displayed not only a high level of repeatability and reproducibility of the cell assay but also an excellent correlation between the endpoint derived from the cell assay and values from standard acute fish toxicity. The present study investigates the applicability of isobolograms to assess mixture toxicity effects in formulated agrochemical products using a cell line assay as an alternative, animal-friendly approach to determine the benefits of this approach for regulatory ecotoxicology and product development.

1.02.P-Th024 Image Based Toxicity Screening of Chemicals in *Daphnia magna*

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Green Chemistry is an essential part of EU's new chemical strategy towards a toxic-free environment. Currently, several tens of thousands of chemicals are found on the global market and a majority of them are not fully tested in terms of ecotoxicity. This requires identification of hazardous chemicals and development of new safe-by-design chemicals for their substitution. The research program Mistra SafeChem is targeting a holistic approach to chemicals and promotes a green chemical industry in Sweden. Chemical developers working together with computational and experimental toxicologist to find green solutions for the future and to predict risks already in the design phase. To assess if a chemical is "safe", appropriate testing approaches that allow efficient generation of toxicological effect data is demanded. High content-screening (HCS) describes automated microscopic image acquisition with subsequent quantitative evaluation of multiparametric data sets. This image-based method is, combined with molecular fluorescence stains and antibodies, a well-established technique to observe cellular processes *in vitro*. To help develop ecotoxicological testing, we are combining the standard test species *Daphnia magna* with HCS. This allows us to identify toxic responses after exposure to chemicals, mixtures or environmental samples and generate molecular mechanistic data while keeping the complexity of a whole organism. Since imaging is a non-invasive method, the physiological structure of the test organisms is maintained which allows acquisition of spatiotemporal information. Application of softwares such as ImageJ facilitates fast image analysis and extraction of light intensity data which are proportional to the toxic response of interest. Intensities can be used as semiquantitative measure of toxicity. The simultaneous application of several stains with fluorescence at different wave lengths enables multiplexed analysis in one test object. Combined with time resolved observation of toxic response this can help to fill knowledge gaps in ecotoxicology and further develop adverse outcome pathways (AOPs).

1.02.P-Th025 From Histology to Behavior: Sublethal Effects of Developmental Neurotoxins in Zebrafish (*Danio rerio*)

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The developing nervous system is extremely vulnerable to exogenous stressors like chemicals. Early life-stage exposure may lead to persistent morphological and behavior alterations. Developmental neurotoxicity (DNT) studies according to OECD TG 426 provide information on neurologic and behavioral abnormalities of rat offspring after exposure to repeated doses of chemicals *in utero*. Given the use of rodents, such studies are extremely time- and cost-intensive and require a large number of experimental animals. As a consequence, although adopted by OECD, data are still scarce, since OECD TG 426 is applied only in cases of strong suspicion of a neurotoxic potential. In contrast, relatively inexpensive experiments with rapidly developing zebrafish (*Danio rerio*) embryos appear as a promising alternative for routine DNT testing. Starting as early as 21 hours post-fertilization, spontaneous tail coiling within the embryonic chorion can easily be recorded to detect behavioral changes. In conjunction with later observations on modifications of swimming patterns, tail coiling recordings may provide valuable insight into manipulations of the developing motoneuron-muscle-system. In order to link behavioral to morphological observations, histological analyses of muscles and motoneurons were made. Thus, the purpose of the present study was to complement various methods to test the neurotoxic potential of chemicals in developing zebrafish embryos with a focus on motoneuron-muscle interactions. Combining behavioral endpoints with histology was hypothesized to provide a hint as to not only the chemicals' targets, but also to adverse outcomes in a complex vertebrate model. Chemicals tested as model compounds were the well-known DNT reference nicotine (positive control) and desnitro-Imidacloprid, a major metabolite of imidacloprid (neonicotinoid) with a higher neurotoxic potency to vertebrates than its parent compound. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002 (EU-ToxRisk; GA, art. 38.1.2).

1.02.P-Th026 Metabolic Products and Toxicity Pathways As Weight-Of-Evidence in the Use of Transcriptomic Points-Of-Departure (tPODs) in Embryonic Rainbow Trout Exposed to Benzo[A]Pyrene

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Current ecological risk assessment frameworks rely on approaches that require large numbers of live animals and lengthy time for chronic responses to manifest, rendering them costly, tedious, and of significant ethical concern. New Approach Methods (NAMs) such as rapid mechanistic embryo assays are not only more ethically acceptable but can also be more efficient by characterizing molecular events, leading to the derivation of thresholds that could guide decision-makers on identifying or prioritizing chemical hazards. However, instituting such an approach requires careful calibration through multiple lines of evidence. Thus, this study aimed to establish a NAM where a transcriptomics benchmark dose (transcriptomic points-of-departure; tPOD) was derived from short-term exposure experiments with non-protected embryonic stages of rainbow trout (RBT; *Oncorhynchus mykiss*) using

benzo[a]pyrene (B[a]P) as the model compound. In brief, embryo-alevin RBT were exposed to graded concentrations of B[a]P (measured: water control, 0.01% DMSO solvent control, 0.079, 0.35, 1.5, 7.4, and 28.6 µg/L) for 28 days. Benchmark dose analysis of toxicogenomic data (RNASeq) at 4 days post-hatch yielded tPODs of 0.02, 0.15, 1.8, and 0.07 µg/L B[a]P for the median of the 20 most sensitive active genes, 10th percentile of all active genes, mode of the first peak of gene-level benchmark doses, and pathway-level (glutathione metabolism pathway) tPOD, respectively. After 28 days, morphometric analysis showed significant growth inhibition at >7.4 µg/L B[a]P, with notable decreasing trend in body weight. Molecular pathways, biochemical responses (EROD, oxidative stress), and histological alterations will be used to construct toxicity pathway models and B[a]P metabolites will be measured to demonstrate similarities in toxicological perturbations and processes occurring at early life and more advanced life stages of fish as reported in the literature. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

1.02.P-Th027 Optimization of Fish Embryo Acute Toxicity Test Workflows in the Promising Fish Model *Oryzias melastigma* for Marine Ecotoxicology

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The marine medaka, *Oryzias melastigma*, has been identified as a promising model species for marine ecotoxicology and shares many advantages for early life stage testing with the well-established freshwater model species zebrafish (*Danio rerio*) such as easy large-scale culturing and a transparent chorion. The present work aimed on optimizing workflows with *O. melastigma* and the particular focus on the adaptation of standardized procedures such as the Fish Embryo Acute Toxicity (FET) test (OECD 236, ISO 15088) and associated endpoints like locomotor activity or heart rate analysis. In this context, general adaptations related to the test conditions and species-specific characteristics such as, e.g., pre-treatment of embryos, sensitive time windows of exposure (max. 22 hpf), test duration (up to 192 hpf), miniaturization to 96-well plates for higher throughput testing as well as different dechoriation approaches, were performed. In addition, the positive control 3,4-Dichloroaniline was adapted and DMSO as well as MeOH were evaluated as potential carrier solvents (recommended concentration ~ 0.1 %). The optimized marine medaka FET protocol was successfully validated with neurotoxic model compounds (e.g. paraoxon methyl, carbendazim) and is currently applied to investigate environmental extracts. For a direct sensitivity comparison to zebrafish, species-specific (e.g. embryonic development, onset of biotransformation) as well as external factors (e.g. medium salinity, temperature) were analyzed. In comparison to the chorion of *D. rerio* (1.5 – 2.5 µm), the thicker chorion of the medaka (15 µm) was identified as a critical point and hence, the role of the chorion was further examined. In this context, also the influence of DMSO on chorion permeability and thus, the compound uptake, was investigated using confocal laser scanning microscopy and compared with existing data for zebrafish. Results support the hypothesis that the medaka chorion provides a stronger barrier compared to the zebrafish chorion and hence could be a major factor affecting the bioavailability and resulting toxicity. Since behavioral analysis is an increasingly important endpoint for the ecotoxicological evaluation of neurotoxicity, also a light/dark transition locomotor response test using neuroactive substances was adapted. Typical swimming patterns as reported for zebrafish were observed, indicating a promising potential of this test for the behavioral assessment of *O. melastigma*.

1.02.P-Th028 Effects of a Native and Chemically Dispersed Light Crude Oil on Multi-Level Endpoints in the Marine Medaka *Oryzias melastigma* Embryo Model

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Adverse effects of crude oil exposure are well-known to manifest even at low concentrations during early development of fish. The present study investigates the applicability of a multi-level endpoint battery in *Oryzias melastigma*, the marine medaka, which is increasingly established as a model organism in marine ecotoxicology. Furthermore, this work addresses the potential changes in resulting toxicity caused by the weathering of crude oil. The fish embryo acute toxicity test (FET) with additional mechanism-specific endpoints on cardio- and neurotoxicity is performed. To evaluate the species sensitivity and applicability as a marine model the present findings will be compared to previous results with the freshwater model *Danio rerio* using identical experimental set ups. Freshly fertilized eggs were exposed to native and chemically dispersed water accommodated fractions (WAF) of a light naphthenic crude oil. WAFs prepared from the dispersant itself and an inert oil were used as controls. WAFs were prepared in embryo medium (25 ‰ salinity) at 10 °C and mixed for 40 h before exposure. Embryos were incubated at 28 °C using a semi-static setup to ensure a steady bioavailability of adverse compounds. The work with *O. melastigma* embryos was performed up to 192 hours post fertilization which corresponds to animal-free testing period. Embryonic development was monitored every 24 h and video sequences were recorded at 120 and 168 hours post fertilization for heartbeat assessment. For further mechanism-specific endpoints on neurotoxicity, oxidative stress and biotransformation activity with enzymatic biomarkers (AChE, CAT, EROD), embryos were exposed to sublethal concentrations (EC5-EC20). In this context, also dechoriation and following swimming behavior alteration tests (light dark transition test) will be performed. First results of the FET showed a concentration-related increase in toxicity and confirmed effects as hemorrhaging, slowed development and decreased heart rate and blood flow. Moreover, cardiotoxicity was shown by evaluation of heartbeat frequency with significantly declined heart rate in WAF exposed embryos. Initial comparison of fresh and weathered oil indicated increasing toxicity after weathering, which will further be examined by chemical analysis of exposure solutions. Overall, the present findings support the applicability of the marine medaka as a relevant and useful model organism for marine ecotoxicology.

1.02.P-Th029 A Review of the Use of the OECD Threshold Approach for Fish Acute Studies

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Fish acute toxicity studies (OECD TG203) are embedded in global regulatory frameworks for chemical risk assessment; however, this study type is ethically controversial as a vertebrate study with a lethality endpoint. Alternative approaches are available (OECD TG236, OECD TG249) but not yet widely accepted by regulatory authorities. Projects are ongoing to support the use of alternative approaches (CEFIC, SWiFT), however, for the meantime, LC₅₀ determinations using OECD TG203 are still required. Fish acute toxicity studies are routinely conducted at Scymaris Ltd. to support global chemical risk assessments for our clients and the OECD threshold approach is used wherever possible. Not only does this approach negate the need for range finding, but it also reduces animal numbers from 42 to 14 fish in most cases. The threshold approach utilises data generated from algae (OECD TG201) and *Daphnia magna* (OECD TG202) acute toxicity studies to set a limit concentration for the fish study. Only if mortality is observed is a full range of 5 test concentrations required. This approach supports the principles of the 3Rs by reducing animal numbers and refinement of the test concentration by utilising data from other test organisms. We conducted a retrospective assessment of the fish acute toxicity studies conducted at Scymaris. The data presented in this poster gives an overview of when the threshold approach has been utilised, the concentrations chosen based on algae and *D. magna* endpoint data and the subsequent numbers of fish used and spared by using this approach. The studies were conducted in compliance with GLP (Good Laboratory Practice) and in accordance with the OECD TG203 or international equivalent.

1.02.P-Th030 Optimization Approach for an Animal-Free High-Throughput Micronucleus Assay

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In vitro bioassays are efficient tools for determining biological effects in environmental samples containing complex mixtures of contaminants. For example, assessment tools are available that predict whether a sample is genotoxic without having to perform a costly and time-consuming animal experiment. Exposure to genotoxic agents can lead to the formation of a phenomenon called micronuclei, which corresponds to the loss of genetic material. Such loss of genetic material can have effects on the cell, the organism, and possibly on the entire population and is, therefore, a major genotoxicity endpoint in standardized *in vitro* assays, such as the OECD Guideline 487 micronucleus test. However, the visual scoring of micronuclei is time-consuming and scorer fatigue can lead to inconsistency. In this regard, more recent technologies like automated scoring, for example, by digital image recording and analysis is a viable, less resource-intensive alternative. According to the OECD Guideline 487 and ISO/DIS 21427-2 2009, the micronucleus assay is originally performed on slides. In this study, the development of an improved high-throughput (HTP) *in vitro* micronucleus assay in multiwell-plates, using an automated imaging microscopy approach to rapidly identify micronuclei is described. Following the 3R principles, we attempted to adapt a transgenic H2B-eGFP V79-4 cell line to a chemically defined medium to reduce the animal-derived components in the assay design. The novel HTP method in 96-well format allows a throughput of 4 samples with 5 concentrations, a negative control, positive control, solvent control, and process control in 4 technical replicates each. In summary, the miniaturized version of the assay, performed in 96-well plates, has several advantages compared to the slide method. Besides increasing sample throughput, reagent reduction and optimized image acquisition were realized. To increase the data throughput of the test and to avoid scorer bias, automatic evaluation is desirable. However, this is limited within the present test design since the nuclei of V79-4 cells do not have a homogeneous appearance and vary greatly in shape and size. Additionally, the reduction of animal components in the test design was challenging and chemical defined media need to be evaluated for the cultivation of cells with round, homogenous nuclei, such as the human A549 cell.

1.02.P-Th031 Effect of Different Sample Preparation Techniques on the Fish Embryo Test (FET): A Comparative Study Using *Danio rerio* and *Oryzias melastigma*

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Fish embryo toxicity tests (FETs) are particularly suitable for the toxicological characterization of environmental samples due to their ecological relevance and high throughput potential. In particular, environmental extracts can be dosed in the FET at different concentration levels to achieve a dose/response relationship for lethal and sub-lethal endpoints. However, an environmental extract needs to go through a series of preparation steps before bio testing. These processes may alter the chemical composition of the sample and lead to an over- or underestimation of the sample toxicity. A comprehensive overview on the effects of different sample preparation procedures on the final outcomes of the FET is still mostly unknown. In this study, we want to compare the effects of different dosing procedures using a freshwater (*Danio rerio*) and marine (*Oryzias melastigma*) fish embryo species. Therefore, an environmental sample collected from an area impacted by greenhouse activities was collected in 2018 using onsite large volume solid phase extraction (LVSPE). The extract was prepared using different enrichment procedures covering two common carrier solvents (DMSO, ME OH) and the direct re-dissolving in exposure medium. Fish embryos were exposed to the extracts following a slightly modified version of the OECD 236. Embryonic development was observed every 24 h and exposure media and organisms were collected for chemical analysis to understand the effect of uptake and substance losses during sample preparation. Preliminary results showed that *D. rerio* has a higher sensitivity than *O. melastigma* (up to 4 fold) to the extract. Furthermore, our results confirmed that different sample preparation procedures are not influencing the FET with *D. rerio*. However, we observed a slightly lower toxicity when the sample was dosed without using carrier solvents ($p < 0.001$). In contrast, different sample preparations influenced adverse outcomes in *O. melastigma* and extracts dosed in methanol induced a higher

toxicity ($p < 0.005$) compared to DMSO, showing the impact of sample preparation on the FET with marine medaka. These results together with a potentially lower sensitivity to environmental extracts in respect to *D. rerio* need to be further interpreted by the chemical analysis of internal and exposure media. Our results underlined the need to achieve a harmonization of sample preparation strategies for testing environmental extracts.

1.02.P-Th032 Comparing Ecosystem and Human Health Damages From Chemicals in Household Products Using Predicted Chronic Hazardous Concentrations

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Empirical ecotoxicological data is only available for a limited number of chemicals and species. Thus, predictive models can be utilized when experimental data is lacking. Linear regression and random forest models were developed to predict chronic freshwater hazardous concentrations (HC20) of the species-level chronic NOAEL for chemicals in the US EPA Chemical and Product Database (CPDAT). When acute HC50 data was available, chronic HC20 were predicted using simple linear regression ($N=4,887$; $R^2=0.89$). To generate predictions for chemicals lacking acute HC50 values, chemical toxicity parameters from Open Structure-activity/property Relationship App (OPERA) and Toxicity Estimation Software Tool (TEST) were entered as inputs to a random forest model that was able to predict close to 60% of the HC20 variability ($N=1,828$; $R^2=0.59$), against only $R^2=0.394$ when performing multi-linear regression. When TEST data was missing for a chemical, chemical properties from OPERA were entered into a separate random forest model ($N=4,251$; $R^2=0.371$). After building and training the three models, the models were used to predict HC20 data for 700,000 chemicals, with the highest performing model being used in priority per chemical. Next, HC20 predictions were combined with population-level product usage data from Stochastic Human Exposure and Dose Simulation Model (SHEDS-HT) and extrapolated to the generic USEtox human population. Human toxicity impacts and HC20-based ecotoxicity impacts were modeled in USEtox, accounting for chemical concentration, fate, and transport in environmental media. Damages were expressed respectively in units of disability adjusted life years (DALY/kg emitted) and potentially disappeared fraction of freshwater species (PDF m^3 d/kg emitted). Total PDF and total DALY were analyzed for each chemical and product. Chemicals with the highest human health damage include triethanolamine, dichloromethane, and hydroquinone. Chemicals with the highest ecosystem damage include cyfluthrin, 2-Methyl-3(2H)-isothiazolone, and ?-cyhalothrin. Outdoor spray insecticides were the most damaging products to ecosystems, while leave-on personal care products were the most damaging to population-level human health. Human health and ecosystem damages differ greatly by chemical and product, underscoring the need for individualized analysis for each type of toxic impact.

1.02.P-Th033 Employing Adverse Outcome Pathways for Identifying Hazards Associated With Novel Flame Retardants

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Organophosphorus flame retardants (OPFRs) have been consistently detected in increasing concentrations in the environmental and human matrices. Growing evidence suggests that numerous health hazards are associated with exposure to OPFRs, including metabolic disease. However, the underlying mechanisms linking exposure with metabolic pathologies remain poorly understood. In the present study, we used human liver cell culture (monolayer and 3D spheroids) to characterize the toxicological effects and potential mechanisms for OPFRs-mediated metabolic disruption. We employed an *in vitro* bioassay toolbox to assess the key events (KEs) in the proposed adverse outcome pathways (AOP) for liver toxicity (hepatic steatosis). We examined nine OPFRs through *in vitro* bioassays for lipid accumulation, mitochondrial dysfunction, expression of several genes related to lipid metabolism and utilized the *in silico* approach to identify the putative molecular initiating events (MIEs). Our findings suggest that the aromatic (aryl-) OPFRs, i.e., tricresyl phosphate (TMPP), triphenyl phosphate (TPHP), 2-ethylhexyl diphenyl phosphate (EHDPP), and the chlorinated OPFR, tris(1,3-dichloropropan-2-yl) phosphate (TDCIPP) induced the lipid accumulation in human liver cell culture by altering the expression of genes encoding for hepatic lipogenesis and mitochondrial dysfunction. Available data from ToxCast and *in silico* molecular docking suggested pregnane X receptor (PXR) and peroxisome proliferator-activated receptor-gamma (PPAR γ) as potential MIEs. Moreover, EHDPP-mediated dysregulation of hepatic lipidome was also observed in human 3D hepatospheroids. Several lipid classes, including sterol lipids, sphingolipids, glycerolipids, glycerophospholipids, and fatty acyls, were disrupted after EHDPP exposure. In summary, our study identifies several OPFRs as a potential risk factor for metabolic pathologies and hazardous for the environment and human health. It also demonstrates the utility of an AOP-based strategy for screening, prioritizing chemicals, and elucidating the molecular mechanisms of toxicity. **Acknowledgments:** Supported by the EU H2020 program under the Marie Skłodowska-Curie grant agreement No. 859891 (PRORISK).

1.02.P-Th034 Effects of 2,4-D Application on Early LIFE Stages of Fish: Bridging Laboratory and Field Work

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Invasive, non-native aquatic species like Eurasian watermilfoil (*Myriophyllum spicatum*) are rapidly spreading across the United States. One common active ingredient used to control this invasive species is 2,4-Dichlorophenoxyacetic acid (2,4-D). Application of 2,4-D to aquatic environments typically occurs while many freshwater fish are spawning and due to 2,4-D stability in the water column of lakes, many non-target species experience prolonged exposure throughout embryogenesis and larval development. Recent laboratory research has shown that ecologically relevant concentrations of 2,4-D can negatively impact multiple species of freshwater fish species at different points during ontogeny, however, it is poorly understood whether these laboratory results accurately predict impacts in the more complex environment of natural lakes undergoing normal practices of 2,4-D application. Therefore, we investigated the effects of 2,4-D whole-lake treatments on the development and survival of fathead minnows in

lakes undergoing treatment using mobile, in situ, and laboratory exposure systems. We also examined how specific water parameters could alter the toxicity of 2,4-D on the development and survival of fathead minnows. Fathead minnow larvae were exposed to either control lakes or lakes undergoing 2,4-D whole-lake treatment and effects on morphology, survival, and growth were evaluated. Our results demonstrate that exposure of fathead minnow larvae to EPA-permitted 2,4-D whole-lake treatments reduced survival as compared to control lakes which was similar to our previous laboratory results, however, the magnitude of the deleterious effects can be altered specific water parameters (e.g. pH). Altogether, our results shed light on the broader environmental costs of 2,4-D application on non-target organisms, which can help inform and improve risk assessment decisions.

1.02.P-Th035 Toxicogenomic Responses Following Early Life-Stage Lead Exposure Precede and Predict Phenotypic Adverse Outcomes in the Amphibian, *Xenopus laevis*

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Current standardised protocols employed to generate amphibian-specific data for chemical hazard assessment are reliant on tests that use a large number of animals. However, there is a gradual shift toward new approach methods (NAMs), including using short-term early life-stage (ELS) exposures coupled with transcriptomic responses to identify specific molecular toxicity pathways that precede and contribute to the adverse effects of chemicals. The objective of this research was to determine if such an approach could identify early toxicological mechanisms of lead (as a model compound and environmentally relevant metal) and predict apical outcomes of ecological relevance for amphibians. In brief, embryos were exposed at 48 hours post-fertilization (hpf) to 70, 210, or 630 µg/L lead nitrate (Pb(NO₃)₂) for three weeks. Tadpoles in the high lead group (630 µg/L) had reduced total body length and increased incidence (46.3%) of developmental abnormalities at the end of exposure. Analysis of toxicogenomic data (RNASeq) after 96 h of lead exposure revealed 328 significantly dysregulated genes including those related to DNA repair, cell cycle, apoptosis, immune function and metabolism. While the phenotypic response (developmental abnormalities) was only seen at the highest lead exposure concentration, there were common dysregulated metabolic pathways at both lead concentrations indicating their mechanistic contribution to the altered growth and developmental abnormalities observed in this study. Results from targeted gene expression analysis conducted using a XL EcoToxChip, a species-specific gene expression array, supported the RNASeq data with high concordance between the two platforms in terms of identified dysregulated pathways and technical performance. This study demonstrates how gene expression profiling can help identify molecular mechanisms of chemical toxicity in ELS amphibians and could be used to screen chemicals with the potential to cause adverse outcomes. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

1.02.P-Th036 Identification and Toxicity Assessment of Potentially Toxic Cyanobacterial Metabolites From Two Brazilian *Microcystis* Strains

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Anthropogenic contaminants seriously threaten aquatic ecosystems, yet, natural toxins represent a permanent risk to the aquatic biota. Cyanobacterial blooms are a worldwide concern due to their ability to synthesize a wide variety of bioactive secondary metabolites (so-called cyanopeptides), and only a few classes have their toxicological potential elucidated - such as the microcystins. Nevertheless, the risk associated with most of the cyanopeptides classes in aquatic environments and drinking water remains overlooked. To explore the risk of cyanopeptides, we used the zebrafish (*Danio rerio*) in early life stages. Larval fish survival and changes in morphology were assessed for 120h exposure to HPLC-purified extracts of two different cyanobacterial species commonly found in bloom episodes in Brazil (*M. aeruginosa* - NPCD-01 strain – and *M. panniformis* - MIRS-04 strain). The cyanobacterial extracts were first fractionated by HPLC, and the fractions analysed by LC-HRMS to identify the set of peptides present in each fraction of each strain. Acute toxicity towards the zebrafish embryos was studied for the pooled HPLC fractions (aiming to study the toxicity of the whole extract) and one single fraction containing a predominant peptide (>90% of total peptides). The Fish Embryo Toxicity tests were performed with the samples dissolved in dilution water, following the OECD Guideline 236 (2013). LC-HRMS of both cyanobacterial extracts showed that the MIRS-04 strain produces microcystins (hepatotoxins) and cyanopeptolins, which are mainly known for inhibiting serine proteases. As for the NPCD-01, a microcystin non-producer, the most abundant cyanopeptides produced by this strain are microginins (proteases inhibitors), with reports of genotoxicity towards *Allium cepa* and toxicity against crustaceans (LC50 values in the low mg/L range). When exposed to the pooled fractions, fish mortality increased with concentration, pointing to acute toxicity for both strains in the same comparable low micromolar range. Fish mortality observed in the experiment with the isolated from the MIRS-04 strain (containing Micropeptin K139) indicates that the toxicity is not only attributed to the presence of microcystins, and this is also proved by the results with the NPCD-01 strain, which was as toxic as the MIRS-04 extract, even though it does not produce microcystins.

1.02.P-Th308 Adverse Outcome Pathway Guided Hazard Assessment of Mitochondrial Uncouplers: A Tiered Testing Strategy Using Zebrafish In Vitro Assays and Embryo Toxicity Assays

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Oxidative phosphorylation (OXPHOS), that takes place in the mitochondria, is the primary metabolic process that produces

energy in the form of ATP. Chemicals that cause mitochondrial dysfunction, by uncoupling OXPHOS can lead to adverse effects of regulatory concern such as growth inhibition. In ecotoxicity assessments the need for transition from traditional animal toxicity tests to alternative testing strategies is encouraged by various chemical regulatory frameworks (e.g. REACH). This study aims to utilize the adverse outcome pathway (AOP) concept and animal alternatives to develop a tiered testing strategy for hazard assessment of mitochondrial uncouplers. For this purpose, a suite of high-throughput zebrafish *in vitro* assays and embryo toxicity assays have been developed, with the guidance from the newly published AOP linking the uncoupling of OXPHOS to growth inhibition, via a reduction of ATP pool and cell proliferation (OECD project #1.92, AOPWiki, AOP #263). The *Danio rerio* embryos, ZF-L and PAC2 cells were used for AOP-guided hazard assessment. Zebrafish cells and embryos were exposed to the known mitochondrial uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP). *In vitro* and *in vivo* assays were conducted to generate temporal and concentration-response data for the key events defined in the AOP. The initial results showed a decrease in coupling of OXPHOS, ATP pool and cell proliferation in zebrafish cells. Anchoring the effects at the cellular level, the growth of zebrafish embryos exhibited a concentration-dependent decrease after 96 h exposure to CCCP. Survival of zebrafish embryos was also affected by CCCP. The bioassays for measuring the earlier key events in the AOP will also be performed and compared with the *in vitro* results. The present study shows that three out of four key events in AOP 263 could be measured using both *in vitro* and embryo assays. The two test systems displayed different sensitivities to the model uncoupler CCCP. The results will be used to develop a tiered *in vitro-in vivo* testing strategy for more efficient assessment of mitochondrial toxicity and the construction of a quantitative AOP (qAOP) based on AOP 263. Chemical analysis will also be performed to support *in vitro* to *in vivo* extrapolation. *Acknowledgement* - This study was funded by the Norwegian Research Council (project 301397, www.niva.no/en/projectweb/riskaop) and supported by the NIVA Computational toxicology Program (www.niva.no/en/projectweb/nctp).

1.02 Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future (Virtual Only)

1.02.V-01 A Novel Approach Using *Throa* α - and *Dre-Mir-499* α - Zebrafish for Screening the Thyroid Endocrine Disruptor

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A number of bioassays are commonly used to detect estrogenic and androgenic chemicals in the environment. However, *in vivo* screening tools to identify thyroid endocrine disruptors are generally limited, and most assays cannot provide information on toxic mechanism of a given chemical. In the present study, we aimed to establish a sensitive and rapid screening tool capable of characterizing the mechanisms of thyroid endocrine disruption. We developed a novel *in vivo* thyroid endocrine disruptor screening system employing wild-type, thyroid hormone receptor alpha a knockout (*thr α -/-*), and *dre-miR-499* knockout (*dre-miR-499-/-*) zebrafish embryo. In addition, we verified the validity of this assay using T3, propylthiouracil, and thiamazole. Thyroid endocrine disruptors were successfully screened based on a comparison of larval survival between wild-type and knockout zebrafish embryo/larvae within 96 h. The combination of our *in vivo* screening assay and subsequent characterizations will be a valuable source of information for thyroid endocrine disruptor profiles. *Acknowledgement*: This study was supported by National Research Foundation of Korea (NRF; Project no. 2019R1A2C1002712).

1.02.V-03 Are Global Standard Test Species Suitable for Environmental Risk Assessment of Crop Protection Products in Tropical Environments?

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World-wide data requirements for ecotoxicological risk assessments require initial laboratory testing of pesticides to assess their toxicity to aquatic species. Clearly not all aquatic species world-wide can be tested. Thus, surrogate species of fish, aquatic invertebrates, and primary producers are tested, using standard protocols, for assessing the risks of pesticides. These surrogate, standard test species, representing the different trophic levels, have been chosen based on several criteria including sensitivity, ecological relevance and availability. However, they were not chosen based on geographical distribution. Therefore, this study evaluates whether these standard test species, already used in many parts of the world, are suitable for use in risk assessments for tropical environments. Data for tropical fish, invertebrate and primary producer species were extracted from the supplemental material by Wang et al. (2019), the USEPA ECOTOX database and peer reviewed literature. For each pesticide, the most sensitive regulatory endpoint for fish, invertebrates and primary producers was selected. The ratios of the endpoint for the tropical species and for the most sensitive regulatory endpoint for the appropriate taxonomic group were determined. Values > 1 indicate that the tropical species are less sensitive than the respective standard regulatory species. In total toxicity endpoints for more than 40 different pesticides on more than 150 different tropical species were collected and assessed. In 96.3% of the evaluated fish data, the tropical fish species were less or similar sensitive (within a factor of 5) than the standard fish species. For the aquatic invertebrates, 93.9% of the evaluated tropical species were less or similar sensitive compared to a standard species. For primary producers, for 96.6% of the evaluated data the toxicity ratio of the tropical and the standard species endpoints resulted in values >1. Overall, in more than 95% of the data comparisons between tropical and standard aquatic species the standard species were more or similar sensitive. In conclusion it can be stated that, for the data set under investigation it was demonstrated that data derived from standard test species were also suitable for use in risk assessments in tropical regions.

1.02.V-04 Avoidance Tests As an Ethical Ecologically Relevant Alternative to Fish and Amphibian Lethality Tests for Chemicals Registration

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Despite recent advancements in the search for alternatives to minimize animal testing (e.g. OECD 249), in the tier 1 environmental risk assessment (ERA) for the registration of chemicals fish lethality tests (e.g. OECD 203) are still among the most widely conducted ecotoxicity tests. Ethically, welfare concerns for animal suffering are self-evidently, as the aim is to determine the concentration required to kill 50% of fish, with large numbers of animals being used. Similar welfare concerns arise concerning amphibian toxicity tests, even if they are not yet implemented at the regulatory level, given that from the five available standard guides for amphibian assays only one (ASTM E1439-98) does not involve animal experimentation (*sensu strictu* do not use life stages with any independent feeding). From the traditional toxicological perspective, these forced exposure tests confining organisms to a constant contaminant concentration are designed to derive precise cause-effect relationships needed to best estimate risks of environmental impairment. However, from an ecological perspective, an unreliable level of risk is most likely produced because many fish and amphibian species are known to detect unfavorable conditions and spatially avoid disturbed habitats. Consequently, natural populations can become locally extinct, before any organism dies, because individuals promptly detect and emigrate. Contaminants may act thus as habitat disturbers regulating fish and amphibian dispersion patterns, by provoking emigration from disturbed habitats; but at chemical concentrations well below those that would be estimated as lethal in tier 1 ERA. To halt animal distress, pain and suffering, while gaining ecological relevance in ERA at the ecosystem and landscape level, as urged by many EU legislation (e.g. Directive 63/2010, REACH), the present study intends to review already existing data on avoidance tests with fish and amphibians exposed to a contamination gradient in a design amenable to standardization; being the ultimate goal to discuss the value of implementing avoidance tests as a tool and ultimately ban lethal testing in the ERA for chemicals. Avoidance tests are thus a key alternative ecotoxicity refinement tool to integrate the 3Rs (Replacement, Reduction, Refinement) policy, as well as the new 3Rs (Reproducibility, Relevance, applicability for Regulatory use), in the tier 1 toxicity tests without compromising environmental protection.

1.02.V-05 *Daphnia magna* As an Alternative Model to Evaluate Toxicity for Eicosanoids Pathway

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Eicosanoids play an important role in inflammation, allergy, fever and immune responses. In the eicosanoids pathway, cyclooxygenase (COX) catalyzes the conversion of arachidonic acid to prostaglandins (PG) and is a crucial target of painkiller pharmaceuticals, i.e., COX inhibitors. Thus, toxicological study on eicosanoids pathway is important for new painkiller drug discovery and also for the evaluation of adverse health outcome by environmental contaminants. However, the experimental models are very limited. This study was to develop an alternative model for evaluating toxicity on eicosanoids pathway. To this end, we used *Daphnia magna* model as alternative model. In this present study, COX inhibitors, such as celecoxib, naproxen and mefenamic acid, were chosen. *Daphnia magna* was exposed to those chemicals for 24 h. The analysis of related gene transcriptions (*COX*, *pla2*, *PGDsyn*, *LTB4dh*, and *LOX*) by qPCR were followed. In addition, the concentration of eicosanoid molecules, such as arachidonic acid and prostaglandins, were analyzed by LC-MS/MS. Multiple reaction monitoring was used for the selective quantification of target molecules. After the exposure, transcriptions of the *LTB4dh* and *COX* genes were up-regulated. In addition, the whole-body level of arachidonic acid which is converted by COX was increased by the three COX inhibitors. From the gene transcriptions and eicosanoid molecules analysis, it is shown that *D. magna* has similar eicosanoids pathway with other vertebrate models. This might show the plausibility of *D. magna* as an alternative model to assess the toxicity of the eicosanoids pathway.

1.02.V-06 Development of LOW Invasive Methods for Biomarkers Assays in Amphibians: Tests on the Saliva

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Worldwide biodiversity is declining at an unprecedented rate with amphibians being the most threatened vertebrate group. A major cause of their decline is the exposure to human related contaminants released into the environment. However, few ecotoxicology articles are published on amphibians each year. Due to their terrestrial and aquatic lifecycle, they can be exposed to many contaminants. This may be the case with pesticides application in fields, if individuals are present, or in the vicinity. It can also occur later, if the molecules or its degradation products are persistent enough to be transported by runoffs to the water bodies. Amphibians can therefore be impacted at all stages of their lives and their permeable skin makes them vulnerable to the absorption of undesirable molecules. Biomarker measurement makes it possible to look at individuals' exposure to contaminants by looking at molecule's activity variations in the organism. For example, the exposure to contaminants can generate a change of detoxifying¹ or regulatory² enzyme's activity and confirm that the individual's body is under stress. In 2017, searchers (Mingo *et al.*) used common wall lizards' saliva to measure biomarkers. They detected an activity difference before and after they were exposed to pesticides. This minimally invasive method follows the 3RS principles which aims to reduce, refine and replace animal use in science. Moreover, it allows for fast sampling with buccal swabs and does not require organs' recovery from euthanised individuals. In the present study, we used amphibians' saliva and liver samples and measured three enzymatic biomarkers (GST¹, EROD¹, AChE²). The experiment was made on *Xenopus laevis* raised in laboratory conditions. These organisms were euthanized for technical reasons and we tried to get as much data as possible from those, following the 3RS rules. We also collected

Ichthyosaura alpestris saliva samples in the field. All biomarkers were detected in liver samples, whereas only GST and EROD were in saliva samples. From this dataset, the aim was 1) to assess whether saliva can be used to predict changes in enzyme activity in the liver which is a key organ for the body detoxification 2) to see if morphological traits (size, weight) influence biomarkers' activity. First results show that some biomarkers can be detected in saliva. Its use could replace more invasive methods when possible and facilitate the sample collection in laboratory and in the field.

1.02.V-07 Dichlorvos (DDVP) Pesticide Toxicity on 3 Species of Fishes

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The pesticide Dichlorvos (DDVP) is an organophosphate compound, used for the control of ectoparasites in fish, considered by the EPA as highly toxic. Because studies on the effects of this product in fish are scarce, the objective of this work was to evaluate its toxicity and their effects on 3 biomarkers: growth, lipid peroxidation (lipoperoxidation) and the activity of the acetylcholinesterase (AChE) enzyme in *Chirostoma jordani*, *Danio rerio* and *Poecilia* sp. Initially, a test with duration of 96 hours was carried out where the juveniles of the different species were exposed to 5 concentrations of the pesticide to determine the lethal concentration 50. Subsequently, a bioassay with a duration of 10 days was carried out where the fish (juveniles) were exposed at 2 sublethal concentrations (LC₁ and LC₅), at the end of the exposure period the biomarkers were evaluated (n = 10). The LC₅₀ values obtained in the toxicity bioassays ranged from 4.82 ± 1.95 mg L⁻¹ to 0.0013 ± 0.00067 mg L⁻¹, *Chirostoma jordani* was the most sensitive species to DDVP. The growth rates of the exposed organisms were between 19 to 75% lower than those observed in the control group. There was a decrease of between 21 to 80% in the activity of the enzyme AChE and the degree of lipoperoxidation in the tissues increased up to 180%. The pesticide used in this work is not very persistent in the environment but the results of this study indicate that its effects in sublethal concentrations were irreversible, because the organisms do not feed.

1.02.V-08 Evaluation of Tissue Integrity and Lipid Distribution in the Liver Tissue of Fish *Poecilia reticulata* After Exposure to Iron Oxide and Glyphosate Nanoparticles

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Iron oxide nanoparticles (IONP) have been investigated as potential tools for environmental remediation, as they are magnetic and present, when functionalized, the surface capacity to adsorb glyphosate, as described in the literature. Thus, it is necessary to evaluate the toxicity of the association of IONP and glyphosate in aquatic organisms. For this purpose, 7 female fish of the species *Poecilia reticulata* were tested in triplicate, exposed for 7, 14 and 21 days to the treatments: 1) ferric chloride (FeCl₃) in 0.3 mg L⁻¹ (IFe) - as a dissolute part of IONP; 2) IONP+GBH1: 0.3 mgFe.L-1 IONP + 0.65 mgGLY.L-1 (GBH); 3) IONP+GBH2: 0.3 mgFe.L-1 IONP + 1.30 mgGLY.L-1; and 4) IONP+GLY: 0.3 mgFe.L-1 of IONP + 65 mg. Glyphosate P.A L-1 (GLY) and a control group (GC) was maintained. After that, part of the animals remained for 7, 14 and 21 days in reconstituted water for recovery. All were euthanized and dissected, liver samples were fixed in 2.5% glutaraldehyde, post-fixed in 1% OsO₄ solution, embedded in Epon 812 resin, sectioned 0.5 μm thick and stained with toluidine blue or hematoxylin for lipid labeling and tissue integrity assessment. In GC animals, lipids were distributed in droplets significantly smaller and more dispersed than in treated ones. On the other hand, in animals treated with IFe, IONP+GLY and IONP+GBH there was the formation of circulatory, regressive and progressive changes that indicated high inflammatory activity during exposure, which was confirmed by the presence of sites of melanomacrophages with lipofuscin zones in large quantities, mainly in the IFe group and due to the increase in the size and volume occupied by the lipid droplets in the IONP+GLY and IONP+GBH treatments, already during the post-exposure the lipid droplets decreased in size over time, and there was a reversal of the inflammatory changes. Coming to the conclusion that exposure to IONP associations with GLY and GBH were hepatotoxic to guppies, but these fish were able to recover after 21 days of exposure. The analysis of cell and tissue biomarkers contributed to the understanding of toxicity, as well as in resilience of aquatic organisms exposed to associations in order to support the development of safe environmental remediation practices.

1.02.V-09 High-Throughput Ecotoxicology With a Rainbow Trout Gill Cell Line

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The United States Environmental Protection Agency's tiered hazard evaluation strategy is currently human-centric and does not include high-throughput (HT) in vitro assays for ecotoxicologically-relevant species. In 2021, the Organisation for Economic Co-operation and Development adopted test guideline 249 (OECD TG249) for cell viability testing in rainbow trout gill cells (RTgill-W1). Adjusting the assay results for in vitro disposition and conducting in vitro to in vivo extrapolation, excellent correlation to in vivo survival data was found. At present the assay is conducted in 24-well plates, which limits throughput. The goal of the present study was to miniaturize the assay to 384-well format while following OECD TG249 to the extent possible. Moreover, imaging-based HT phenotypic profiling (HTPP, 'Cell Painting') will be conducted in parallel to obtain information about sub-cytotoxic bioactivity of chemicals. RTgill-W1 cells were successfully expanded. One day after plating in 384-well format, media was exchanged, and cells treated with chemicals. After 24 h of exposure, viability stains (alamar blue, CFDA-AM, neutral red) were applied and measured using a plate reader. HTPP plates were labeled to visualize seven different cellular structures, followed by imaging and quantification. In a preliminary experiment, the positive control chemical 3,4-dichloroaniline affected cell viability at

500 uM, but phenotypic changes were already apparent at 5 uM. The established assay will be used to screen a set of 231 environmental chemicals. Of those, 128 have available rainbow trout in vivo toxicity data, 60 have been used in rainbow trout in vitro experiments, 110 have been tested in the HTPP assay in human U-2 OS cells and 29 were detected in the Great Lakes. For all chemicals, nominal in vitro points-of-departure (POD) will be estimated. Adjusting for in vitro disposition and applying HT toxicokinetics, this POD will be extrapolated to chemical concentrations in water used for in vivo exposures. For 12 compounds, chemical concentrations found in the different compartments will be analytically measured. To conclude, a low cost, HT test system suitable for screening large libraries of chemicals for cytotoxicity and phenotypic effects to fish gill cells was established. This expands the current first tier of the hazard evaluation strategy with inclusion of the first ecologically relevant species. *This abstract does not reflect USEPA policy.*

1.02.V-10 Investigations on Fish Acute Toxicity of Fragrance Ingredients, Involving the Chinese Fish Species and the Zebrafish Embryo

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While toxicity data with zebrafish (*Danio rerio*) have been routinely used and accepted worldwide for evaluating the risk of chemicals to aquatic vertebrates, the recent Order of the Ministry of Ecology and Environment of China now requires to generate fish toxicity data in a local laboratory and with the local species Chinese rare minnow (*Gobiocypris rarus*). This represents an additional regulatory constraint that may result in redundant testing, additional animal uses and costs since the Chinese rare minnow is not part of OECD recommended fish species. A recent review concluded for this dataset that juvenile Chinese rare minnow is more sensitive than the zebrafish juveniles and embryos, especially to metals and that tests with zebrafish embryos could serve as an alternative for the juvenile fish acute toxicity test across fish species. In order to understand the application to fragrance ingredients, we have selected 29 fragrance ingredients belonging to various chemical classes and with a variety of physicochemical properties, for which good quality zebrafish acute toxicity data were available and tested them according to the OECD TG 203 with juvenile *G. rarus* and to the OECD TGD 236 with embryo *D. rerio*. Both chemical toxicity distribution (CTD) and chemical ratio distribution (CRD) models were established to systematically compare the sensitivity among juveniles of *G. rarus* and *D. rerio*, as well between *D. rerio* embryos and juveniles. The results of CTD models showed that for the fragrance ingredients tested, the sensitivity of juvenile *G. rarus* was similar to that of juvenile and embryos of *D. rerio*. In addition, the similar sensitivity of juvenile and embryos of *D. rerio* to fragrance ingredients confirms the suitability of replacing juvenile with zebrafish embryos. The CRD comparisons reveal that juvenile *G. rarus* was less sensitive by a factor of ~2 to juvenile *D. rerio* for ingredients belonging to the Verhaar-Class 3 and ECOSAR-Esters class. The outcome of this comparative experiment supports so far our earlier literature review that concludes the fish toxicity data with *G. rarus* can be used in chemical registrations outside China, and that there is no scientific justification to repeat animal tests.

1.02.V-11 Is a Water Control Needed in Fish Early Life Stage Toxicity Studies Using Solvents?

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A team of scientists in the European Union and the United States led by the PETA Science Consortium International e.V. has been exploring whether both water (negative) and solvent controls are needed in aquatic studies that use solvents. A database of toxicity endpoints for multiple species and solvents has been evaluated for systematic differences between the water and solvent controls and to develop representative dose-response curves. Extensive computer simulations were conducted to determine the potential impact on ECx estimation and NOEC values of using only the solvent control in fish early life stage (FELS) toxicity studies that use solvents. The design of the simulation study drew on previous research by numerous authors with regard to the effects of solvents at the levels allowed in FELS studies. The collected database comprises a minimum of three species of fish (Fathead Minnow (*Pimephales promelas*), Sheepshead Minnow (*Cyprinodon variegatus*) and Rainbow Trout (*Oncorhynchus mykiss*)) and three solvents (dimethylformamide (DMF), acetone, and triethylene glycol (TEG)). The study explored all response variables required in FELS toxicity studies including hatching success, time to hatch, time to swim-up, larval survival (intermediate and total) and abnormalities, and fish length and weight at study end. Computer simulations covered the observed ranges of variability (including heterogeneity or overdispersion) and concentration-response shapes in each type of response across species. The study explored both model selection and model averaging for ECx estimation including standard normality-based models and generalized non-linear mixed models. Standard normality-based hypothesis tests (Williams and Dunnett, Cochran-Armitage), non-parametric methods (step-down Jonckheere-Terpstra and Dunn) and generalized linear mixed models provided NOEC values; the analysis adjusted both NOEC and ECx for overdispersion or variance heterogeneity. Analysis of each simulated dataset included (a) pooled water and solvent control, (b) water control only, and (c) solvent control only, to give side-by-side comparison of results (NOEC or ECx) using the three choices for control(s). The findings from this work will inform whether two controls are necessary for FELS studies.

1.02.V-12 Morphological and Functional Characterization of Brown Trout Primary Hepatocyte Spheroids As a Tool for Toxicological Studies

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Monolayer cultures obtained from fish primary hepatocytes have been used either to test the mechanistic effects of chemicals or to

assess compound's toxicity in the liver. Three-dimensional (3D) systems have emerged as models that can better mimic *in vivo* conditions, an obvious advantage over traditional two-dimensional (2D) cell cultures. In fish, the establishment of 3D hepatocytic models is not as developed as in mammals. Therefore, there is still a long way to go towards expanding these models to more fish species and to better characterize those 3D cultures. In this study, it was established a long-term culture of primary brown trout hepatocyte spheroids obtained from 1 year-old brown trout (*Salmo trutta fario*) juveniles by using a two-step collagenase perfusion procedure. The aim was to obtain a 3D fish hepatocytic model with a well-defined timeline characterization in terms of morphology and functionality. Brown trout primary hepatocyte spheroids were maintained in culture along 25 days post-isolation at 18 °C, without additional supply of O₂/CO₂ and at constant agitation (± 100 rpm). Hepatocytes were plated at a density of 5×10^5 cells/mL in 6-well microplates using 3 mL of DMEM/F-12 with 15 mM HEPES, 1% of antibiotic/antimycotic solution and 10% charcoal FBS. Sampling was performed at days 8, 12, 16, 20 and 25 post-isolation. At each time point, a defined number of spheroids were photographed under a light microscope and biometric parameters such as diameter, area and sphericity were obtained using the AnaSP software. Hepatocyte viability was checked in all sampling days by using lactate dehydrogenase and resazurin assays. To evaluate the general hepatocyte morphology, spheroids were fixed in formaldehyde, submitted to a routine histological processing and stained with hematoxylin and eosin. Quantitative real time-polymerase chain reaction (RT-PCR) was used to monitor the potential changes in the mRNA levels of a selection of functional target genes, namely in terms of hepatic metabolism and transport. Our study contributes to a better comprehension of morphology and functionality of brown trout primary hepatocyte spheroids allowing a more detailed knowledge of this model with great potential to be used in toxicological studies. **Funding:** Supported by FCT and ERDF (UIDB/04423/2020 and UIDP/04423/2020) and ICBAS.

1.02.V-13 Species and Life Stage Sensitivity of Chinese Rare Minnow (*Gobiocypris rarus*) to Chemical Exposure: A Critical Review

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Chemical production and consumption in Asia are increasing at an unprecedented rate, calling for advancing regulations for chemical management. Under the New Chemical Substance Notification in China, information on ecotoxicological effects of chemicals is mandatory for the simplified registrations of chemicals with the requirement that one ecotoxicological test is conducted locally. It is now mandatory to use the native fish species, Chinese rare minnow (*Gobiocypris rarus*). However, its chemical sensitivity compared to that of standard fish species, such as the fathead minnow (*Pimephales promelas*) or zebrafish (*Danio rerio*) is still unclear. Here, we performed a holistic literature review on fish toxicity data from 1997 to 2020. Species sensitivity among *G. rarus*, *P. promelas* and *D. rerio* and life stage sensitivity of *G. rarus* were systematically investigated for various chemicals using both chemical ratio distribution and probabilistic chemical toxicity distribution approaches. Comparatively, the Chinese native fish species, *G. rarus* was more sensitive than *D. rerio*, particularly to metals. Juvenile and adult *G. rarus* were more sensitive than its larva and embryos. The observed lower sensitivity of *G. rarus* embryo was likely due to the thick embryonic chorion, discrepant methods to collect embryos and paucity of toxicity data, implying the necessity to standardize *G. rarus* embryo tests and validate the sensitivity with various types of chemicals. This unique review allows to conclude that *G. rarus* studies could be used in worldwide registrations and that further investigations are needed to use *G. rarus* embryos as alternatives to the fish test.

1.02.V-14 Sublethal Effects of E-Cigarette Vaping Liquid on Zebrafish Larvae: A Preliminary Study

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The use of electronic cigarettes as an alternative to the conventional tobacco cigarette is becoming increasingly frequent even among teenagers and pregnant women. Vaping devices are, among those available on market, more appreciated by many users for their supposed non-toxicity and the possibility to smoke without tobacco. E-cigarette vaping liquids and aerosols actually contain a mixture of many different substances such as diethylene, glycol, formaldehyde, metals and other volatile organic compounds such as benzene and toluene, which could potentially have adverse effects on human health. Furthermore, the massive use of e-cigarettes can also give effects to aquatic ecosystems. The aim of the study is to investigate the potential effects of the e-cigarette liquid with the use of zebrafish. In particular, zebrafish embryos have been exposed to different concentrations of the liquid and to a flavour separately. Several endpoints were considered to evaluate the different modes of action of the substances, such as morphological parameters (teratogenicity), the measurement of heartbeats (cardiotoxicity), the coiling activity tail (CAT) and the locomotor behavior (neurotoxicity). All parameters have been recorded and evaluated through the use of specific softwares. The results underline how the exposure to vaping liquids is cause of several developmental effects in zebrafish embryos, and neurotoxicity has been detected at different concentrations of the products. The study also confirms zebrafish as a good test organism for its sensitivity and versatility. More studies are needed to better understand the toxicity of this electronic devices and in order to protect human health and ecosystems.

1.02.V-15 Towards High Throughput Determination of Biotransformation Rates of Chemical Mixtures Using Isolated Perfused Trout Livers

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Bioconcentration factor (BCF) is an important criterion in chemical risk assessment that describes the bioaccumulation potential of chemicals. While BCF as determined from *in vivo* whole-fish exposures is still considered the gold standard to inform this criterion, there is growing concern in academia, governments, and industries about the suitability and reproducibility of this test,

especially for chemicals that are biotransformed. Alternative approaches using *in vitro* biotransformation assays based on hepatocytes or liver sub-cellular fractions in combination with *in vitro-in vivo* extrapolation (IVIVE) models have been developed as potential replacements. However, extrapolation to BCF is complicated by confounding factors, e.g., extrahepatic biotransformation and quality issues with experimental BCFs. Therefore, there is a need for an *ex situ* model at an intermediate level of biological organization. A recently developed method is that of the rainbow trout isolated perfused liver, seeking to reduce uncertainty of extrapolated clearances of chemicals in IVIVE. The present study seeks to obtain hepatic clearance data of five environmental contaminants of interest within the isolated perfused trout liver and cross-validate with prior standardized *in vitro* methods. Livers of sexually immature rainbow trout were cannulated *via* the hepatic portal vein and perfused for up to six hours with a physiological buffer spiked at varying concentrations of pyrene, phenanthrene, 4-*n*-nonylphenol, deltamethrin, and methoxychlor. Afferent and efferent samples were taken in 15-minute intervals across the perfusion period. Samples were analyzed using high-performance-liquid-chromatography with fluorescence detection (HPLC-FLD) and gas-chromatography (GC) to calculate hepatic extraction fraction and clearance. Results demonstrate that this experimental method can be used to validate IVIVE models, as illustrated by the excellent fit of predicted versus measured hepatic clearance values. This study has the potential to settle an important debate in this field and enables scientists to focus on other factors to allow for confident predictions of bioconcentration in fish.

1.03 Computational new approach methods (NAMs) supporting regulatory decision making for chemical safety (Part I)

1.03.T-03 The Source to Outcome Predictor (STOP) - a Conceptual Tool for Next Generation Risk Assessment (NGRA)

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Knowledge of how single chemicals and their complex mixtures affect organisms, e.g., their mode of action (MoA) and how these perturbations translate into adverse effects (outcomes), is continuously increasing due to extensive bioassay testing activity worldwide. However, generation of such effect data, typically by bioassays ranging from classical (eco)toxicological tests to New Approach Methodologies (NAMs), is rapidly generating disparate data sets that are not easily integrated. Efforts to link MoAs to adverse outcomes at individual and population levels using Adverse Outcome Pathways (AOP) lends a great promise to facilitate such integration, and developing computational approaches to harvest AOP-informed data and conduct quantitative evaluations for mechanistically informed hazard and risk assessment are thus highly warranted. Integration of AOP knowledge, quantitation of the event relationships across the AOP continuum (quantitative AOPs), developing *In vitro* to *In Vivo* Extrapolations (IVIVE) and interpretation of these in terms of ecological exposure relevance are thus quickly becoming a bottleneck for successful use of an ever increasing amount of data. This applies in particular to the transition from single chemical assessment to cumulative hazard (CHA) and cumulative risk (CRA) assessments of “real world” exposure scenarios, where a high degree of complexity reigns and standardised approaches are still lacking. The present work aims to demonstrate how a Source-To-Outcome Pathway (STOP) framework, taking advantage of the Aggregated Exposure Pathway (AEP) to address complex exposures and AOPs to provide weight-of-evidence-based definition of causal relationships between stressors and adverse outcomes across different levels of biological organization, can be supportive of Next Generation Risk Assessment (NGRA). The chosen approach takes advantage of the AOP knowledgebase to identify relevant AOPs for a given exposure scenario and use this to select relevant bioassays and toxicity data from different levels of biological organisation for subsequent use in hazard and risk assessment. A case study representing typical assemblies of priority pollutants and compounds of emerging concern was used to demonstrate the utility of the approach and how an end-user friendly Graphical User Interface can be used to support NGRA. Acknowledgements: RCN 268294 “MixRisk”, and NCTP: NIVA’s Computational Toxicology Program, NCTP (www.niva.no/nctp).

1.03.T-06 Free In Silico Tools to Predict Multiple Properties and Activities by QSAR

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In the last 20 years, our research group has developed many validated QSARs for the prediction of different endpoints relevant to support the hazard and risk assessment of heterogeneous organic chemicals. As the number of these QSARs increased over time, it became evident that new tools were needed to ease their dissemination and application not only amongst experts, but also among users that are not necessarily QSAR experts. This work is the result of a process, which combines our experience in software development with the most recent progresses of our research in the field of toxicokinetics and PBT profiling. Since 2018 four software have been developed by our research group: QSARINS-Chem standalone version, QSARINS-Chem ECO.44, IVBP-Suite and QSAR-ME Profiler. They have been developed using the Java™ language thus allowing their execution on different platforms like, for example, Windows™, Mac OS™ and Linux. Both the two QSARINS-Chem versions allow for a detailed analysis of individual models and of the predictions generated for multiple endpoints. The *in vitro* Biotransformation Prediction Suite (IVBP-Suite) is dedicated to the prediction of *in vitro* intrinsic hepatic clearance in mammals using reactivity based QSARs and complements the QSARINS-Chem ECO.44 software. IVBP-Suite calculates predictions of multiple models in batch and provides combined output. Finally, QSAR-ME Profiler further expands the concepts of QSARINS-Chem ECO.44 and IVBP-Suite by pooling their functions as a single package. It additionally includes the automatic detection and analysis of chemical structural similarity, for a better profiling of the chemicals of interest. These software, developed to be complementary in their functionalities, have been designed to be user-friendly considering both their graphical interface and the steps to follow to apply the QSARs. Furthermore, they simplify and streamline the application of our QSARs, which are provided in bundle, and can be applied to generate single or combined predictions. QSARINS-Chem standalone version, QSARINS-Chem ECO.44 and IVBP-Suite are freely downloadable from the internet (<http://dunant.dista.uninsubria.it/qsar/>). QSAR-ME Profiler is currently under development and will be available soon on the same website.

1.03.T-07 MechoA Profiler Recently Integrated in the OECD QSAR Toolbox: What Can It Do for You?

Franklin Bauer, Carole Charmeau-Genevois, Pascal Bichere and Paul Thomas, KREATiS, France

The MechoA (Mechanisms of Toxic Action) scheme is a structural alerts model to predict the molecular initiating events (MIE) and some subsequent key events for a wide domain of species, chemical groups and mechanisms. The first version of MechoA scheme has been published back in 2018 and is regularly updated. The main utility of MechoA scheme is to reliably define category of substances for read-across approach and develop QSAR for (eco)toxicity endpoints, based on a common mechanism of toxicity. To improve its ease of use for such applications and enhance its visibility, the MechoA scheme has been integrated as a new profiler in the OECD QSAR Toolbox (QTB) as a free downloadable add-in, named iSafeRat® Mechanisms of toxic Action profiler. A few case studies will be taken during the presentation, showing how to install and use the MechoA profiler in the QTB, and showing that it can highlight both similarities and differences that cannot be easily captured using conventional profilers. To make a good use of the MechoA profiler, it is important to understand the meaning of each MechoA class, which will be explained in the presentation. MechoA alone gives the information that you could get only by running many other profilers (e.g. Protein binding + oxidative phosphorylation uncoupling + MOA classification by Verhaar / OASIS). It is also much more informative than using the most commonly used classifications, i.e. Verhaar scheme, because it provides: (i) Information on the mechanisms that happen in different species, (ii) Some basic information about the metabolism of substances, (iii) A specific text to explain the MechoA for each single prediction, which can give mechanistic clues to explain the MIE behind (eco)toxicological effects, (iv) references to more recent literature and knowledge than old profilers. There are also some limitations with this first version: It is currently slow to run, but we are working on a more efficient version. Moreover, because of the way the QTB itself is designed, MechoA profiler is not handy to use as a subcategorization, because in this step, you cannot easily remove or add categories to be kept in 1 or 2 clicks. We are discussing with the QTB management group for the improvement of this functionality. We are also working on the integration of the alerts of Sapounidou et al. (2021) in the MechoA scheme to achieve an even more complete scheme, with a better coverage of aquatic toxicity mechanisms.

1.03.T-08 A Comparison of Mode- and Mechanism-Based In Silico Methods to Classify Environmental Toxicants Leading to an Improved Classification Scheme

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Classification schemes are vital to assign chemicals appropriately to optimise the use of quantitative structure-activity relationships (QSARs) and read-across to fill toxicity data gaps. Well-used schemes, such as Verhaar and Russom, perform such classification on the basis of broad modes of action. Recently, novel schemes, notably those from MechoA and Sapounidou, have refined the process through a greater mechanistic interpretation of toxicity. Whilst information on all schemes has been published, there has yet to be a systematic assessment of the merits of the schemes. The aim of this investigation was to compare the schemes for classification in terms of coverage, mechanistic relevance and applicability to risk assessment scenarios, in addition to looking for opportunities to improve and integrate the schemes overall. The schemes were evaluated against over 5,550 chemical structures. The Verhaar and Russom schemes were run in the OECD QSAR Toolbox (v4.4) and ChemProp respectively. MechoA was run as a standalone platform and has been integrated into OECD QSAR Toolbox v4.5. The Sapounidou scheme was coded into 183 SMARTS strings representing specific, reactive and narcotic mechanisms of action and run in a KNIME Workflow. There was reasonably good concordance between the classifications from the different schemes. The Verhaar and Russom schemes showed the lowest levels of coverage (for Russom all compounds were classified as narcotic if not classified to another mechanism, however many compounds were found to be out of domain). The mechanistically-based schemes have greater coverage of reactive and specific alerts as well as providing direct access to knowledge and meta data. For instance, where possible, the Sapounidou scheme classification is based on the Molecular Initiating Events derived from the relevant Adverse Outcome Pathway (AOP). Knowledge of the AOP will enable greater use of weight of evidence through the application of NAMs to support approaches such as read-across. The outcome of the comparison led to the decision that the two mechanistic methods should be merged, which is part of an ongoing project.

1.03 Computational new approach methods (NAMs) supporting regulatory decision making for chemical safety (Part II)

1.03.T-01 From Causal Networks to Adverse Outcome Pathways

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A decade ago, adverse outcome pathways (AOPs) have been put forward as a tool for organizing toxicological knowledge across different levels of biological organization, from the initial interaction of chemicals with the biological system (MIE = molecular initiating event) to the individual and population level effects relevant for environmental risk assessment (AO = adverse outcome). As of August 2021, only around 400 AOPs have been submitted to the AOP-wiki. There is therefore an urgent need to accelerate the development of new AOPs. Here we present a new approach for development of AOPs, based on a thus far AOP-untapped toxicological resource – causal toxicological networks (CTN). Here, we show a semi-automatic pipeline to transform CTNs into a set of candidate AOPs, and develop two new neurotoxicity AOPs (AOP 399 and AOP 410 on the AOP wiki). Weight of evidence analysis shows that AOPs developed in this way feature high biological plausibility for the key event relationships (KER), while usually lack evidence for key event (KE) essentiality and empirical evidence necessary to transform them into quantitative AOPs.

1.03.T-02 EcoToxXplorer: A Web-Based Platform for Comprehensive Toxicogenomics Data Analysis

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Toxicogenomics data are likely to play a key role in the transition from traditional to alternative toxicity testing methods. The costs of acquiring such data continue to drop, and an ever-increasing number of researchers and regulators would like to get involved in the world of toxicogenomics. However, these data are complex, and analyses typically require advanced programming skills and a deep knowledge of statistics and genomics resources, and as such, are usually handled by expert bioinformaticians. The objective of this project was to design EcoToxXplorer (www.ecotoxxplorer.ca) as a next-generation bioinformatics tool that is high performance, intuitive, and universally accessible to handle transcriptomics data for the purpose of chemical risk assessment and environmental management. The primary EcoToxXplorer analysis pipeline was built for analyzing qPCR data measured with custom EcoToxChip arrays from six ecological species (model organisms: Japanese quail, fathead minnow, African clawed frog; native species: double-crested cormorant, rainbow trout, northern leopard frog). The pipeline includes steps for QA/QC, filtering and normalization, differential analysis, interactive functional analysis, and report generation. The functional analysis is tox-focused, including integration with the AOPwiki and our custom EcoToxModule gene sets that were designed for high-level interpretation of toxicogenomics data. EcoToxXplorer can also process FASTQ files through a Galaxy server and microarray/RNA-seq counts tables through a NetworkAnalyst interface for the same six ecological species of regulatory relevance in North America. The current version of EcoToxXplorer is the result of continuous development since 2017 by the EcoToxChip project team. The development was organized around design-thinking principles in that we iteratively presented the tool to various user groups from academia, government, and industry for testing, and then refined it based on their feedback. This study was conducted as a part of a large-scale Genome Canada-funded project (EcoToxChip project - www.ecotoxchip.ca).

1.03.T-04 Using Genome Resources to Understand Invertebrate Pesticides Sensitivity and Mixture Responses

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Understanding why some species are more sensitive than others to a given chemical is perhaps the most fundamental challenge in ecotoxicology. In this talk, we will highlight how the growing volume of genome and transcriptome data can be mined to identify the nature of the toxicokinetic and toxicodynamic traits that govern sensitivity. Using examples from comparative studies with arthropods and earthworms, we will show how sensitivity can be attributed to the genome complement of species, the presence of key residues in receptor binding domains, and, for the first time, on tissues specific expression patterns. We generated whole genomes and tissue specific transcriptomes for multiple terrestrial annelid and arthropod species. These genomic resources provided a basis for investigating the potential drivers of species sensitivity in three case studies of differential sensitivity between annelids and arthropods for the organophosphate chlorpyrifos, among earthworm species for the neonicotinoid imidacloprid and of differential mixture effects between azole fungicides and pyrethroid insecticide for both taxa. Bioassays identified cases of differential sensitivity between different earthworms and arthropods. Genomic approaches then allowed us to investigate the bases of these differences. 1. Chlorpyrifos. Earthworms (*Lumbricus rubellus*) showed an approximate order of magnitude lower sensitivity to chlorpyrifos than a soil arthropod (*Folsomia candida*). This difference could be linked to tissue specific cholinesterase orthologue expression. 2. Imidacloprid. A large-magnitude (>30-fold) difference in sensitivity for imidacloprid between earthworms. This difference can be explained by difference the expression of different isoforms of sub-unit of the nicotinic acetylcholine receptor that contain amino acid residue associated with strong or weak imidacloprid binding. 3. Mixtures. Difference in the degree of synergy found between different mixtures in arthropod and earthworms. This difference has been linked to the potential for one chemical to affect the toxicokinetic of another by inhibiting phase one and two metabolism to different extents.

1.03.T-09 Integrating Informatics to Broaden the Taxonomic Domain of Applicability for Adverse Outcome Pathways

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The adverse outcome pathway (AOP) framework provides a means to capture existing knowledge about a biological pathway and understand the evidence for causal linkages from molecular level perturbations to apical outcomes of regulatory concern. Typically, AOPs are developed considering available knowledge from a limited number of model organisms, with generalized comments regarding potential extrapolation to other species within similar taxonomic groups (e.g., all vertebrates). However, to gain confidence in the extrapolation of the AOP to other species the Organisation for Economic Cooperation and Development (OECD) Users Handbook recommends evaluation of structural and functional conservation across the levels of biological organization [1]. Advances in bioinformatics allow for broadening the taxonomic domain of applicability (tDOA) to provide evidence of structural conservation across certain key events (KEs) and key event relationships (KERs) that include protein interactions. Additionally, existing data from available literature can be systematically extracted to provide functional evidence for pathway conservation. In instances where the conservation of the KEs or KERs are determined primarily by traits or characteristics of the organism (e.g., presence/absence of a particular organ/tissue; colony structure or lack thereof) a decision tree can be used to examine tDOA capitalizing on various publicly available databases that capture such species-specific information.

Results from the US Environmental Protection Agency's Sequence Alignment to Predict Across Species Susceptibility tool (SeqAPASS; seqapass.epa.gov/seqapass/) have been used to demonstrate how such computational approaches can inform the tDOA, further expanding the utility of the AOP framework to build evidence of conserved pathways across taxa. Coupled to SeqAPASS, systematic methods have also been applied to gather lines of evidence for functional conservation [2, 3]. Together these approaches strengthen the weight of evidence for tDOA in the AOP framework and provide users of AOPs confidence for broader application across species in the context of research and decision-making. The challenge is integration of these approaches in a streamlined manner for rapid input in the AOP-Wiki (<https://aopwiki.org/>). Through demonstrated application of these approaches for AOP development centered on tDOA, data has been generated to inform such integration.

1.03 Computational new approach methods (NAMs) supporting regulatory decision making for chemical safety (Poster)

1.03.P-We001 Strengthening Skin Sensitisation Predictions Combining Enhanced MechoA and In Silico Skin Penetration Predictions

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The iSafeRat® Mechanism of toxic Action (MechoA) Profiler (included in OECD Toolbox 4.5) is an *in silico* model based on structural alerts which predicts Molecular Initiating Events (MIE). Among these, certain MechoA classes detect electrophilic parent substances and metabolites. These direct- and/or pro- or pre-reactive substances are responsible for hapten-protein complex formation as the MIE, a necessary, but insufficient, predictor of the adverse outcome pathway (AOP) for skin sensitisation (SS). In this study, MechoA, which was previously tested for its performance to identify Skin Sensitisation for the substances listed in Cosmetics Europe skin sensitisation database (CESSD) is combined with other *in silico* tools, notably a skin penetration model, to boost the already good level of accuracy previously observed. In the first study, a raw comparison of predictions for SS based on MechoA classes, a good correlation was observed between chemicals triggering MechoA 3 and/or 4.3 and/or 4.4 and positive results observed for SS in the experimental studies. The probability of this positive prediction being correct was 62% relative to human clinical data and of 57% relative to LLNA data. Positive predictivity relative to *in vitro* methods was between 49 – 78% of which, MechoA 3 only correlated with 49% DPRA positives. A strong correlation was observed between negative results identified in SS studies and chemicals with a MechoA prediction that doesn't include MechoA classes 3, 4.3 and 4.4. The probability of these negative predictions being correct was 83% relative to human clinical data and 78% relative to LLNA data. Negative predictivity relative to *in vitro* methods was between 57 – 95%. Chemicals triggering MechoA classes other than MechoA 3 correlated with 74% of negative results in DPRA. Therefore, MechoA can be used to reliably predict non skin sensitiser (78 – 83%), and to predict with moderate reliability (57 – 62%) potential skin sensitisers based on the protein-binding mechanism. In this study we present results of the statistical gain in specificity and sensitivity of SS predictions for substances and metabolites by including the iSafeRat® dermal absorption model. Other improvements to detect more subtle electrophilic groups associated with mild skin sensitisation are also presented.

1.03.P-We002 Development and Validation of a Tiered Assessment Approach Towards Prediction of Acute Toxicity Within the Fathead Minnow

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It is widely accepted that the manner through which assessment of chemical safety has traditionally been performed – that is, by means of costly and animal-intensive *in vivo* protocols – is increasingly outmoded. Despite desire to facilitate eventual replacement of such procedures, development of alternatives acceptable for adoption within a regulatory setting has proved challenging. Whilst many New Approach Methodologies (NAMs) are available, both *in vitro* and *in silico*, general confidence in the capacity of any one technique to adequately reflect corresponding *in vivo* hazard remains low. The derivation of verdicts based upon the weight of evidence sourced from a variety of appropriate NAM, as opposed to reliance upon individual outcomes, is proposed as a means through which uncertainty in judgment may be reduced. This concept has been formalised in the “tiered assessment” approach, whereby a sequential testing strategy covering collections of related NAMs is applied. Previously, we reported the construction of a tiered assessment pathway describing acute oral lethality within the rat – employing statistical techniques in order to derive probability estimates that compounds may hold membership within each of a series of GHS categories describing extent of toxic potency (defined by LD50 range). Within the present study, the approach is extended to prediction of acute ecotoxicity: specifically, the regulatorily-relevant endpoint of lethality towards the fathead minnow (*Pimephales promelas*) following 96-hour exposure. Adopting GHS aquatic toxicity directives, categorical representations of acute hazard (incorporating four classes) were assigned to greater than 600 organic substances holding appropriate *in vivo* LD50 data. Compounds were subject to three tiers of NAM screening: the first a structure-based assessment scheme intended to identify potential mode-of-action. A second stage integrated a battery of *in silico* methodologies, drawing both from existing and novel QSAR models – prior to a third and final round which sought to incorporate supporting *in vitro* data. Following consideration of results emerging from each tier, probability that a substance might belong to each of the four GHS categories was computed by means of ordinal logistic regression. Comparison to corresponding *in vivo* assignment allowed contribution of each NAM grouping to be assessed, backed by a quantitative expression of confidence.

1.03.P-We003 Using Species Toxicity Information to Improve the Predictive Ability of QSAR Models

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Ideal hazard assessment would capture the toxicity of chemicals and pollutants for all organisms living in a contaminated

environment. As this is not feasible, the hazard for untested species needs to be estimated from the data generated for a subset of surrogate species. In practice, assessment factors are applied to protect potentially more sensitive species and to account for the uncertainty that arises from extrapolating from standard lab conditions to the environment. Robust protection of the environment is thus achieved by a combination of experimental toxicity information for surrogate species and the application of a safety factor. This approach relies on the assumption that the observed effects for a surrogate species can predict the adverse outcome for non-tested species, at least to some degree. If this is true, the addition of species level hazard information should improve the predictive performance of quantitative structure-activity relationship (QSAR) models. To test this, we developed QSAR models for different target species and chemical stressors and compare the predictive performance of models including only traditional chemical traits (e.g. molecular fingerprints and physico-chemical properties) with models that include additional toxicity information for other tested species. We use hazard data provided by the US environmental protection agency via the ECOTOXicology Knowledgebase and focus on the prediction of acute LD50 and LC50 values. These values were combined with relevant chemical traits and available species toxicity measures, including LD50/LC50 values and EC50 values for other non-lethal effect endpoints. Machine learning models were used for the predictions and the importance of single traits and trait groups (chemical traits vs. toxicity traits) were assessed. Overall, our findings support the trait-based approach, and we argue that hazard information for non-focal species can be interpreted similarly to chemical traits and can add to the predictive ability of QSAR models.

1.03.P-We004 The Online Freely Accessible Tool for Similarity Screening to Identify Chemicals of Potential Concern

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Screening and prioritization of chemicals is essential to ensure that available evaluation capacity is invested in those substances that are of highest concern. To facilitate the identification of substances of potential concern, we recently developed structural similarity models that evaluate the structural similarity of substances with unknown properties to known Substances of Very High Concern (SVHC) [Wassenaar et al. 2019; <https://doi.org/10.1016/j.comtox.2019.100110>]. Substances that are structurally similar to known SVHCs might be selected for further evaluation, as a high resemblance in chemical structure could be an indication of comparable effects ('similar property principle'). Separate similarity models have been developed for three groups of SVHCs, including SVHCs with (1) carcinogenic, mutagenic or reprotoxic properties (i.e. CMR), (2) persistent, bioaccumulative and toxic, or very persistent and very bioaccumulative properties (i.e. PBT/vPvB), and (3) endocrine disrupting properties (i.e. ED). These models showed promising performance statistics and appear to be more robust than expert judgements. Upon obtaining more experience with the application of the similarity models, we identified several methodological aspects that could be further optimized to improve the performance of the models. Particularly, the PBT/vPvB model misclassified many substances due to amongst others insufficient consideration of the type and number of halogenated fragments and aromatic structures, the SVHC-categorization insufficiently reflected the current SVHC status, and the binary nature of the prediction limited the interpretation of the results. Therefore, the current study aims to improve the performance of the models by (1) separating the known SVHCs in more specific subgroups, (2) by (re-)optimizing similarity models for the various SVHC-subgroups and (3) by improving interpretability of the predicted outcomes by providing a confidence score. In addition, the underlying reference dataset of SVHC substances was updated. The improvements as described in this study are directly incorporated in a freely accessible web-based tool, named the ZZS similarity tool: <https://rvszoekstelsysteem.rivm.nl/ZzsSimilarityTool>. Accordingly, this tool can be used by risk assessors, academia and industrial partners to screen and prioritize chemicals for further action and evaluation within varying frameworks, and could support the identification of tomorrow's substances of concern.

1.03.P-We006 Deriving Baseline Toxicity QSARs for Ionizable Organic Chemicals by Using Experimental and Computational Membrane-Water Partition Coefficients

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Quantitative structure-activity relationships (QSARs) for predicting (eco)toxicity provide a viable alternative for *in vivo* testing in chemical safety assessment. The majority of published QSARs derived for non-specific toxicity (narcosis) rely on the octanol-water partition coefficient (K_{ow}) as a proxy for the lipophilicity of chemicals. Non-specific toxicity (narcosis) is driven by critical accumulation in the phospholipid membranes, resulting in disturbance in their integrity and function. Biological membranes are ordered and anisotropic 3-dimensional structures. Therefore, the main limitation and criticism of octanol being used as a surrogate for lipophilicity is that being a homogenous isotropic media, it cannot adequately describe membrane interactions of chemicals such as ionisable organic compounds (IOCs). Due to their amphiphilic nature, surfactants pose unique challenges with regards to the determination of K_{ow} due to their tendency to accumulate at the octanol-water interfaces and exhibit electrostatic, in addition to hydrophobic, interactions. The membrane-water partition coefficient (K_{mw}) provides a more biologically relevant and methodologically defensible potential alternative to K_{ow} and has been previously shown to perform as well as K_{ow} for predicting the toxicity of neutral narcotics. It also provides a prospective alternative for IOCs and surfactants both in predictions of environmental partitioning processes and toxicity. In this work, we explore the derivation of QSARs for (eco)toxicity of these "hard to predict" chemicals, particularly concentrating on cationic, anionic and zwitterionic surfactants for which K_{ow} is difficult to determine. We will present initial QSARs developed using the K_{mw} values, based on both experimental and computational approaches, combined with the literature toxicity data. Considering that experimental determination of K_{mw} can be complex, time-

consuming and costly, we also explore the application of two computational methods for deriving K_{mw} , based on coarse-grained simulations and COSMOmic calculation, with experimental data serving as their validation sets.

1.03.P-We007 An In Silico Membrane Model for the Screening of Gastrointestinal Absorption of Chemicals of Environmental or Occupational Concerns

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Parallel Artificial Membrane Permeability Assay (PAMPA) has been proposed as a new approach method (NAM) to screening the effective permeability (P_e) of chemicals through the gastrointestinal tract to help effective regulatory decision making for chemical safety. While PAMPA has gained advocacy for its application towards pharmaceuticals, its use for chemicals of environmental or occupational concerns is still rare. Since chemicals of environmental or occupational concerns differ from pharmaceuticals in hydrophobicity and volatility, there is a need for a systematic understanding of mass transfer in PAMPA, if we seek to expand the applicability of PAMPA. We develop a computational model to simulate the mass transfer of chemicals in laboratory PAMPA for mechanistic insights into P_e measurements. The model's performance is evaluated by an agreement between predicted and measured permeabilities of 1383 chemicals. The evaluation shows that the model provides reasonable or conservative estimates for 91% of chemicals in the evaluation set, within a short period simulation time of fewer than five seconds, indicating that the model is efficient and protective of human gastrointestinal absorption of chemical substances. The model predicts an inverted U-shaped dependence of P_e on the octanol-water partition property ($\log K_{OW}$ for neutral compounds and $\log D_{OW}$ for ionizable compounds), with the maximum P_e occurring in $\log K_{OW}$ or $\log D_{OW}$ ranging between 0 and 2. The model estimates a high membrane retention rate for hydrophobic chemicals, as well as the loss of volatilization to the headspace of the PAMPA apparatus for highly volatile chemicals. Notably, the measured permeabilities of hydrophobic chemicals are remarkably sensitive to specific experimental conditions, e.g., frequency of stirring, and incubation time, making P_e measurements under different conditions less comparable. More important, for highly hydrophobic chemicals, steady-states mass transfer, which is the fundamental assumption of PAMPA can never be achieved. In conclusion, our in-silico model not only has the power to predict the P_e values of PAMPA accurately and rapidly. The use of our developed model can not only help cost-effective decision-making for tens of thousands of chemicals in commerce but also predict the permeability for chemicals whose P_e cannot be experimentally determined due to constraints of hydrophobicity or volatility.

1.03.P-We008 Developing High-Throughput Methods for the Hazard Assessment of Chemicals and Particles in *Daphnia magna*

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Standard experiments for the toxicity assessment of single substances towards the keystone species *Daphnia magna* are time-consuming and labour-intensive: acute immobilization tests for the determination of LC50 values take 48 (OECD test no. 202); in life history tests, *Daphnia magna* females are exposed to the test substances for 21 days and daily checks are required (OECD test no. 211). These tests are essential tools for the regulatory risk assessment in aquatic environments, as mortality and reproduction are important toxicity endpoints that likely affect population dynamics. Due to the time-consuming design, the number of chemicals that can be tested with these experimental setups is however limited and can neither account for the large number of substances found in contaminated environments, nor for the large number of candidate substances in product development. In addition, pollutants such as micro- and nanoplastic particles (MNPs) cannot be tested in one-pollutant-one-test approaches, because they are too complex. Each MNP has a unique set of properties, e.g. plastic type, size, shape, age, coating, additives, and therefore assigning MNPs to discrete classes is difficult. To allow for a risk assessment for small scale particles a large number of different MNPs has to be tested to create datasets that are large enough for predictive modelling approaches. We address these experimental constraints of standard test procedures by developing high-throughput methods for the evaluation of *Daphnia magna* swimming behaviour and heart rate measurements. Changes in the swimming behaviour of daphnids are early indicators of stress and later on – immobilization. Heart rates change rapidly in response to stress in general, both resulting in shorter total experimental times. To reduce the manual lab work, we use video recordings and an automated video analysis pipeline combined with automated object detection based on machine learning methods. We show that these methods are less time-consuming and at the same time are good proxies for traditionally derived LC50 values and reproduction effects. We argue that the implementation of high-throughput methods for the evaluation of quick stress responses is beneficial in situations where large amounts of chemicals or pollutants have to be tested within reasonable time periods. We will discuss our approach as a valuable alternative to currently used methods without substantial loss of information.

1.03.P-We009 Development of the Exposure and Safety Estimation (EAS-E) Suite Platform to Integrate New Approach Methods and Data for Chemical Assessments

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Environmental and human health risk assessments require quantitative exposure data for thousands of chemicals in various systems. Understanding the processes that determine chemical exposure provides critical insights for risk management decisions for the safe and sustainable use of chemicals by society. Multi-media mass-balance fate, toxicokinetic (bioaccumulation), and exposure models are often necessary for exposure and risk estimation. These mechanistic (process-driven) models require chemical-specific input data such as physical-chemical properties, toxicokinetic data, environmental degradation half-lives,

production volumes and emission rates. However, for many of the chemicals requiring evaluation, these data are limited, not standardized and documented across multiple databases. To reduce animal use and address significant data gaps, New Approach Methods (NAM) like in vitro bioassays and validated Quantitative Structure Activity Relationships (QSARs) can be used; however, their application often requires expert judgement and a careful evaluation of the results (e.g. Applicability Domain). Most importantly databases and models are not integrated; chemical evaluators need to gather information from many diverse sources to perform assessments. Here we introduce the Exposure And Safety Estimation (EAS-E) Suite platform to bridge the gap between evolving scientific research and regulatory assessment challenges. EAS-E Suite is a free on-line platform developed to facilitate knowledge translation to various stakeholders on issues in exposure assessment and environmental health. EAS-E Suite is comprised of chemical information databases, QSARs for predicting chemical information if measured data are unavailable, and models and tools to aid chemical assessment decision-making. EAS-E Suite provides opportunities to address regulatory challenges for new and existing chemical assessments for ecological and human health objectives to support a One Health approach to chemicals management and to guide the safe and sustainable production and use of chemicals in society. A case study illustrates how the platform integrates various data sources and knowledge across multiple disciplines to reduce uncertainty, improve chemical management and support decision-making.

1.03.P-We010 Risk Assessment of Honey Bee Stressors Based on In Silico Analysis of MOLECULAR Interactions

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The honey bee *Apis mellifera* is an important pollinator and has a fundamental socio-economic role in Europe's food production. Throughout the last decade, a decline of this species has been observed globally. The decline of honey bee colonies is thought to be linked to several stressors, including pesticides and other chemicals used in agriculture. The current risk assessment of pesticides in Europe is focused on lethal effects and lacks reflection on sublethal effects. A better understanding of the effects that exposure to pesticides produce on honey bees is still needed. In this context in silico methodologies such as docking analysis of toxic chemical ligands and proteins could be used as a first step to identify affinity and possible interactions at the molecular level. Currently, dozens of 3D structures of proteins from *Apis mellifera* are present in online databases including odorant-binding proteins, pheromone binding proteins, beta-synthase and protein from the immune system. This study aims to investigate in silico interactions of bee proteins and pesticides to produce a risk assessment analysis that can enhance the protection of honey bees in Europe. For this purpose, a protein structures dataset for *Apis mellifera* was obtained from protein data banks and extended with proteins of homologous organisms. Similarly, the 3D structures of the EU authorised pesticides were obtained from online databases. The molecular docking analysis was performed with AutoDock vina. For each protein from honey bees, a ranking based on the affinity of chemicals for specific and non-specific binding sites on the macromolecule was developed. The possible effects of the identified interaction (protein-toxic compound) were investigated through a literature search. This was linked to potential effects on reproduction, communication, fly and spatial orientation among others. Also, documents (reports) from international organizations, such as EFSA or FAO, and monitoring data were collected to obtain a picture of the sites affected by increased bee mortality. These results were related to the results obtained from the ranking-based affinity. Finally, a risk assessment analysis of the identified molecular stressors of honey bees was performed. The results of this study are considered a starting point to identify new sources of possible stress for honey bees and thereby contribute to the overall understanding of the honey bee decline.

1.03.P-We011 Characterizing Uncertainty in Computational New Approach Methodologies (NAMs) for Chemicals Risk Evaluation: A Typology Framework

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New approach methodologies (NAMs) that employ computational prediction of risks of chemicals aim to support chemical safety evaluation to protect human and environmental health. A major challenge in using computationally driven NAMs is uncertainties, such as in the quality of input data and model accuracy. Decisions based on the prediction outcomes of NAMs can be misleading if associated uncertainties are not well characterized and communicated. This work aims to identify and qualitatively characterize uncertainties related to the development, use, and outcome of recent computational NAMs studies. We identify and characterize uncertainties in the input parameters and variables, data gaps, model prediction, and output and outcome interpretation. Guided by the characterized uncertainties, we propose an uncertainty typology framework and demonstrate contexts where the framework can be applied. Our study indicates that explicit uncertainty characterization and the use of a typology framework are critical steps to ensuring transparent and reliable predictions of the potential risks of chemicals using NAMs.

1.03.P-We324 Improving Ecotoxicity Assessment Results by Splitting Species Sensitivity Distributions (SSD)

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Characterizing ecotoxicity effects, whether as integral part of chemical safety assessment, life cycle impact assessment, or environmental quality contexts, requires to address differences across chemicals in their potential to cause harm on ecosystems. Improving ecotoxicity effect assessment needs digitalization methods and big data analysis to characterize chemical impacts on ecosystem health accurately. There are ~10,000 chemicals with available measured in vivo data, but we still lack a systematic understanding of the different effects of chemicals on specific species groups and the relevance of such understanding on ecotoxicity effect quantification. To address this gap, we introduce a systematic approach for linking chemicals to effects on specific species groups via (a) mapping chemicals to chemical classes, toxic modes of action (MoA), and target species groups,

and (b) combining mapping results with quantified species sensitivity distributions (SSD) for specific species groups. Based on our results, we aim to determine chemicals for which we need to split SSDs as suggested earlier to address more vulnerable species groups and improve the accuracy of ecotoxicity effect factors. As a starting point, we considered and curated experimental data for freshwater species in our analysis from the original database, following a semi-automated approach. SSDs were then built and uncertainty quantified based on intra-species variability from reported test data extrapolated to chronic EC10 equivalents across all species and species groups per chemical. For comparing species groups per chemical and targeted MoA, we used the 20th percentile (P20) point on each SSD. When comparing single and split SSDs per chemical, we could identify two main groups of chemicals. The first group shows non-overlapping uncertainty 95% confidence interval (CI) ranges around their split SSDs, indicating that particular species groups are significantly more sensitive to exposure for the concerned chemical than other species groups. The second group of chemicals shows either overlapping 95% CI ranges around their split SSDs, or split SSDs that cross each other due to differences in slopes, indicating that chemicals are not affecting particular species groups more than others. These results suggest that splitting SSDs can increase the reliability of chemicals that inhibit a specific MoA in certain species groups.

1.03 Computational new approach methods (NAMs) supporting regulatory decision making for chemical safety (Virtual Only)

1.03.V-01 A Novel Molecular Modeling Framework to Predict Protein-Ligand Interactions to Detect Endocrine Disrupting Potential of Chemicals

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There is a need for new approach methodologies (NAMs) to identify substances with an endocrine disrupting (ED) modality. Our goal is to provide an accurate, high-throughput, expandable and customizable tool for molecular modeling that can be used to detect potential ED modality. The interaction of chemicals with proteins is a key to investigate the disruption of endocrine pathways. Among the computational methods that could investigate such interactions, molecular docking (Mol. Dock.) is a technique that has a reasonable tradeoff between computational investment and accuracy of predictions. In the scientific process allowing for such a prediction to deal with the level of statistical complexity presented by the problem, at midterm Mol. Dock. is used as a kernel around which a sophisticated framework must be built. Arguably, there is currently no publicly available “gold standard” computational framework entirely fit for purpose to handle this model, hence, our team is building our own computational framework. Fundamental parts of the framework consist of third-party software, while the linking of software and certain analysis strategies were developed entirely by our team. Our framework under active development, it has open architecture allowing for new datasets, new features, and new methodologies to easily be incorporated, leading to a vast potential for customization and for collaboration. To date our tool can investigate 6 ED EATS targets with a total of 96 protein conformations, this application domain and the span of the conformational space will be extended in the future. This poster presents the latest version (2.0) of the framework, introduced as a novel tool for molecular modeling and analysis of protein-ligand complexes. Furthermore, the application of the framework is illustrated with a case study investigating the binding propensity of a set of chemicals (including some with suspected ED potential) towards biological targets from the EATS modalities.

1.03.V-02 A Quantitative Index to Incorporate Transcriptomic Data Into Weight-Of-Evidence Approaches for Ecological Risk Assessment

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The rapid computational improvement in ecotoxicogenomics analyses, alongside their technological affordability, has brought new insights into the adverse effects of chemicals on aquatic organisms. In the field of ‘omics’ technologies, there is a growing interest towards transcriptomics which investigates genomic-scale changes in RNA expression, indicating both temporary and persistent genome alteration. In this work, a methodology is presented to quantitatively elaborate transcriptional data obtained from aquatic organisms exposed to chemicals with the aim of integrating this information into a Weight of Evidence approach to support ecological risk assessment. The methodology makes use of results from the application of Gene Set Enrichment Analysis (GSEA, Subramanian et al., 2005) to recent studies investigating the response of *Mytilus galloprovincialis* and *Ruditapes philippinarum* exposed, under controlled laboratory conditions, to environmentally realistic concentrations of contaminants, i.e. pharmaceuticals, herbicides, per- fluoroalkyl substances, and fragrances. A set of mathematical algorithms was designed to translate the severity of transcriptional alterations in exposed organisms into a quantitative hazard index. The degree of de-regulation of gene sets organised into higher biological themes together with the relevance of each biological theme in terms of physiological reactions contribute to the calculation of the hazard index. The outcome, expressed on the scale 0-100%, is classified according to five hazard classes (from absent to severe), providing an evaluation of the early individual effects of chemical exposure. The application to the experimental datasets proved that the model can effectively discriminate different levels of adverse transcriptomic response when compared with expert judgement. This methodology can serve as a proof of concept for the integration of “genomic tools” in ecological risk assessment based on multidisciplinary investigations. To this end, the proposed quantitative “transcriptomic” hazard index can in future be incorporated in a quantitative Weight of Evidence approach, where the integration with results from other types of analysis (lines of evidence, LoEs), such as chemistry, bioavailability, ecotoxicological bioassays, benthic communities and cellular biomarkers, can contribute to elucidate the role of chemicals in adverse ecological effects.

1.03.V-03 Development and Analysis of Toxicity Screening Models Based on ToxCast Data Using Various Machine Learning Algorithms and MOLECULAR Fingerprints

Su-yong Bea and Jinhee Choi, University of Seoul, Korea, South

Machine learning and deep learning approaches have been increasingly used in various fields including toxicology. High-throughput screening (HTS) is used to produce large amounts of biological data. The ToxCast program uses HTS to screen biological activity to suggest potential toxic effects. The ToxCast program conducted 1474 in vitro ToxCast assays using over 9227 chemicals. In this study, we used 799 ToxCast assays to train activity predictive models and the data was curated through the following conditions: For each assay dataset, 30 models were trained by combining 6 algorithms (Gradient Boosting Tree, Random Forest, Multi-layered Perceptron Network, k-Nearest Neighborhood, Logistic Regression, Naive Bayes) and 5 molecular fingerprint representations of the chemical (MACCS, Morgan, Layered, RDKit and Pattern fingerprint). The process was carried out using the KNIME platform. The synthetic minority oversampling technique (SMOTE) was used to balance the data, and accuracy and F1 score were used as evaluation criteria of model performance. As a result, 799 models were selected, and 35 models with good performance were selected based on F1 score and accuracy. Finally, models predicting similar results for chemicals with similar structures were classified. Since biological activity data is relatively small in quantity and difficult to mass-produce, it is important to utilize and study existing HTS data to help address these problems. We present 35 models and expect that our result can be used as a cornerstone for a wider range of in silico toxicity prediction research.

1.03.V-04 Identification of Human Diseases Associated With Household Chemicals Using Comparative Toxicogenomics Database

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Household chemical products provide substantial benefits to human life, so their use is steadily growing in modern societies. However, the misguided use of household chemicals can potentially cause significant harm to human health, as demonstrated in the terrible incident in Korea (2011), where biocidal chemicals were used as disinfectants for household humidifiers. This tragedy substantially showed that more rigorous toxicity assessments covering various uses of chemicals is essential to household chemical product safety management. In this study, to gain insight into the human health implications of chemicals found in household products, human diseases associated with household chemicals were identified using the Comparative Toxicogenomics Database (CTD, <http://ctdbase.org/>). For CTD analysis, permitted chemicals listed in the Korean ‘Act on the Safety Management of Household Chemicals and Biocides’ were analysed, and we selected five use categories (Coating, Detergent, Disinfection, Air freshener and Insect repellent) according to the number of chemicals. Representative chemicals (Sulfuric acid, Phosphoric acid, Propane, Kerosene and Ethanol) for each use category were selected based on volume used, and were used to collect chemical-induced disease, associated gene and phenotype information from CTD. For each use category and their representative chemicals, we suggested a priority list of chemical-induced diseases and associated genes and phenotypes. This study emphasizes that analysis using a database with comprehensive toxicity information can support and facilitate further studies on priority settings for management of potentially exposed household chemicals. Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through ‘Environmental Health R&D Program’ (2021003310005).

1.03.V-05 Integrating -Omics Data With Benchmark Dose Analysis to Develop an Adverse Outcome Pathway for Hepatocellular Carcinoma

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Allyl alcohol is an allylic alcohol used as a precursor in the manufacture of plasticisers, pharmaceuticals, resins, acrolein, military poison and herbicides. Inhalation exposure to ally alcohol can occur via degradation of chemicals found in e-cigarette vapor. Allyl alcohol undergoes alcohol dehydrogenase (ADH) oxidation to form acrolein leading to hepatotoxicity and eventually hepatocellular carcinoma (HCC). In this study we present an adverse outcome pathway (AOP) for HCC. An AOP is a theoretical framework that integrates existing knowledge to describe the effect of a substance from its initial interaction with a biological system (molecular initiating event) cascading through the different levels of cellular, linked together by key event relationships required to produce a toxic effect (adverse outcome), in this case, HCC. We use benchmark dose (BMD) analysis to identify the pivotal point at which key events in the AOP occur. The BMD is the dose that causes a predetermined change in the response rate of an adverse effect. In this study we extracted human gene expression data of allyl alcohol exposure from Open TG GATES (<http://toxico.nibiohn.go.jp/english/>), calculated the BMD and mapped the values onto genes and biological processes to identify the important processes initiated at these values. Disease and biological process data were collected from the Comparative Toxicogenomic Database (<http://ctdbase.org/>) to build the AOP. We found that the pathways leading to HCC were time and dose sensitive. The most sensitive process leading to HCC at 2hr exposure is “intrinsic apoptotic signaling pathway in response to nitrosative stress” via DDIT3 modulation eventually activating “cell cycle arrest” and “inflammatory response”. “Endothelial cell activation involved in immune response” was activated via TCIM modulation, leading to “cell migration”. At 24hr exposure, we found “negative regulation of non-canonical Wnt signaling pathway” via modulation of RNF213 leading to “epithelial to mesenchymal transition”. “Negative regulation of protein kinase C signaling” via modulation of MYADM lead to “apoptosis”. We suggest an AOP for HCC based on the modulation of DDIT3, TCIM, RNF213 and MYADM.

1.03.V-06 Artificial Intelligence-Based Toxicity Prediction of Environmental Chemicals: Future Directions for Chemical Management Applications

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Recently, research on the development of artificial intelligence (AI)-based computational toxicology models that predict toxicity without the use of animal testing has emerged, because of the rapid development of computer technology. Various computational

toxicology techniques that predict toxicity based on the structure of chemical substances are gaining attention, including the quantitative structure-activity relationship. To understand the recent development of these models, we analyzed the databases, molecular descriptors and fingerprints, and algorithms considered in recent studies. Based on a selection of 84 papers published since 2014, we found that AI models have been developed to predict approximately 30 different toxicity endpoints using more than 20 toxicity databases. For model development, molecular access system and extended-connectivity fingerprints are the most commonly used molecular descriptors. The most used algorithm among the used machine learning techniques is the random forest, while the most used algorithm among deep learning techniques is a deep neural network. The use of AI technology in the development of toxicity prediction models is a new concept that will aid in achieving a scientific accord and meet regulatory applications. The comprehensive overview provided in this study will provide a useful guide for the further development and application of toxicity prediction models. Acknowledgment: This work was supported by a grant from the Korean Ministry of Environment through 'Environmental Health R&D Program' (2021003310005).

1.04 Environmental Epigenetics: Short-term and long-term effects and challenges for both ecotoxicology and human toxicology (Poster Corner)

1.04.PC-Mo01 Integration of Epigenetics Into Multi-Omics Analysis Reveals Alterations in Neurotransmitter Signalling in Zebrafish Embryos Exposed to Tributyltin

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Environmental exposures to endocrine disruptor chemicals (EDCs) have been proven to significantly affect the epigenetic status of ecotoxicologically relevant species of fish, plants, and invertebrates. However, the mechanism of action remains unclear. For that reason, further studies are needed to better elucidate how EDCs act on epigenetic endpoints capable to regulate gene expression. In zebrafish, a recognized toxicological model species, environmental epigenetics in addition to other omic layers including transcriptomics and metabolomics have been applied to explore holistic molecular perturbations by a great variety of pollutants. Nevertheless, each type of omic data is specific to a single "layer" of biological information, impeding a comprehensive understanding of toxicity mechanisms and key events for adverse outcome pathway development. Therefore, the integration of multi-omics analysis seems to be a powerful tool to efficiently capture, not only the toxic mechanisms but also multifaceted networks from gene to phenotype. In the present study, we have exposed zebrafish eleutheroembryos from 2 to 5 days post-fertilization from 3 to 100 nM of TBT. Whole genome bisulfite sequencing revealed that DNA methylation was affected in a dose-response manner, affecting genes related to neurotoxicity. Results from RNA sequencing showed a general, rather unspecific toxicity pattern at higher doses of TBT. This was in concordance with morphometric results previously published. Steroid biosynthesis pathways that lead the synthesis of cholesterol and vitamin D₃ appeared as the modules that become affected at the lowest TBT concentrations. Last, untargeted LC-HRMS metabolomic analysis demonstrated lipid disruption and alterations in taurine and energy metabolism. Results achieved in the multi-omic pathway enrichment were in concordance with the main single omics results. Interestingly, the multi-omic analysis also disclosed that pathways related to neurotransmitter signalling were also significantly affected by TBT exposures. Behavioural testing revealed alterations in behaviour, not only at the end of exposure but also three days after embryos were transferred to clean water. Our results suggest that DNA methylation alterations in neurotransmitter signalling could provoke long term behavioural effects. Altogether, demonstrates the usefulness of multi-omic data analysis to better understand the modes of action of EDCs.

1.04.PC-Mo02 Transgenerational EpiGenetic Effects After Early LIFE Stage Exposure to Perfluoroalkyl Substances

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Research shows that the individual lifestyle of an organism can influence its phenotype and maybe also the phenotype of its offspring. The different genetic and non-genetic components of the inheritance system and their mutual interactions are key mechanisms to generate inherited phenotypic changes. Once released into the environment, pollutants represent a serious threat to human and environmental health and their exposure may also affect subsequent generations through transgenerational epigenetic modifications. Thus, there has been growing concern that epigenetic modifications by pollutants mediate adverse health outcomes over several generations. But only a limited number of environmental studies have been published about the effects of pollutants on epigenetic marks and related adverse outcomes and even less investigate changes of epigenetic marks over generations. Poly- and perfluoroalkyl substances (PFAS) are particularly investigated due to long and extensive use in firefighting foams, textiles, and paper products. Among PFAS, perfluorooctane sulfonic acid (PFOS) is the most studied as it is known to be persistent, bioaccumulative, toxic, and undergoes wide transportation across all environmental media. Perfluorobutane sulfonic acid (PFBS) is considered less toxic and is now used in replacement for PFOS. However, preliminary results indicate that PFBS alters epigenetic marks; thus, further investigations are needed to study the impact of PFBS on organisms over several generations. Therefore, we will study the transgenerational epigenetic inheritance of PFAS to increase our understanding of how these compounds impact epigenetic mechanisms and related adverse effects. For that purpose, we will induce a precocious (0-2hpf post-fertilization) and chronic (28 days) exposure of two PFAS: PFOS and PFBS, on zebrafish embryos. Then we will study the impact of this exposure on zebrafish transcriptome, metabolic activity, phenotype, and behavior on the exposed generations (F1) but also

on the subsequent unexposed ones (F2-F3), to investigate the transgenerational effect of PFAS. Finally, we will look at the epigenetic and genetic components to decipher the molecular mechanisms involved beyond the transgenerational effects of PFAS.

1.04 Environmental Epigenetics: Short-term and long-term effects and challenges for both ecotoxicology and human toxicology (Virtual Only)

1.04.V-01 DNA Methylation and Epigenetic Biomarkers for Xenobiotic Exposure in Mediterranean Fin Whales

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Mediterranean cetaceans are increasingly exposed to a plethora of anthropogenic impacts, including the pressure exerted by chemicals pollutants. Among the different effects, as a result of the exposure to environmental pollutants, epigenetic modifications, such as DNA methylation (DNAm), can occur and subsequently to affect and regulate gene expression. Hyper- and hypo-methylated genes on fin whale (*Balaenoptera physalus*) skin biopsies sampled in the Pelagos Sanctuary (North – Western Mediterranean Sea) have been identified by DNAm profiling through reduced representation bisulfite sequencing (RRBS) in organisms with different contaminant loads. The expression of a panel of genes (hypo-methylated genes: CCDC93, SETD1A, SMARCA2, PKP3; hyper-methylated genes: OTX1, PCBP3, CLSTN1, SHANK1, GRAMD4) were quantified by droplet digital PCR (ddPCR). The variations in the expression of the selected genes, which are mainly involved in cell differentiation and functioning of vascular and nervous systems, were evaluated according to the sex of organisms and the contaminant (plastic additives) load measured in the blubber of the same individuals to evaluate the effects of environmental stress on DNAm and, thus, on gene silencing or transcriptional activation. The up- and down regulation of panel of hyper- and hypo- methylated genes revealed a correlation between the levels of contaminants and the expression of the selected genes. These results provide a first insight on the potential use of a panel of biomarker genes related to epigenetic modifications, which can be used as prognostic markers for genetic adaptation of a vulnerable species to a changing environment in such a contaminated environment as the Mediterranean Sea.

1.05 Investigating endocrine-disrupting properties of chemicals: developments and challenges towards new approach methodologies (NAMs) (Part I)

1.05.T-01 Endocrine-Disrupting Potential of Sediment in South African Harbours

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South Africa is surrounded by a coastline of about 3000 km. There are eight main ports in South Africa which include the ports of Mossel Bay, Cape Town, and Richards Bay. Richards Bay and Cape Town can be classified as industrial ports, Cape Town being the second busiest port in the country. Mossel Bay is a recreational harbour. Tributyltin commonly used in harbours have been known to cause endocrine disruption. In this study sediment sampled from these three harbours were analysed to determine if there is any (anti-)androgenic activity. The MDA-kb2 human breast cancer cell line is genetically modified with the mouse mammary tumour virus (MMTV) luciferase neo reporter gene construct. The MDA-kb2 cells have endogenous androgen and glucocorticoid receptors (AR and GR respectively). The MMTV reporter gene is activated when ligands bind to the AR or GR—resulting in the expression of luciferase. The sediment samples were extracted with 2:1 hexane:acetone and evaporated to make up 1 mL of concentrated extract in methanol. The cells were exposed to the samples (0.02–5 g/mL) in triplicate. Reference compounds testosterone (T) (1.6–85 ng/mL) and flutamide (F) (2.1–500 µg/mL) were dosed in the activation and the inhibition assay respectively. The light emitted by the samples were compared to the standard curves of the reference compounds and expressed in terms of T or F-equivalents. Agonistic effects were found at four of the total samples (n=12) indicating the presence of likely AR agonists. Richards Bay 1 represented the highest activation with 135%, with a BEQ₅₀ of 1.9x10⁻⁹ ng T-eq/g sediment. Half of the 12 samples caused an antagonistic response with no evidence of cytotoxicity. Most of the sites in the Richards Bay area inhibited the AR. The BEQ₅₀ of the quantifiable samples ranged from 6.93x10⁻⁶ µg F-eq/g–7.85x10⁻⁵ µg F-eq/g. Evidence of agonistic effects was observed during the inhibition assay: 6 of the 12 samples, with Cape Town 5 having an activation of 168%. This is likely due to a synergistic effect between the extracted compounds and testosterone. No cytotoxicity was observed for the samples with quantifiable luminescence response: cell viability was higher than 60% in the MTT viability assay. The compounds extracted from the samples indicated AR activation and inhibition. The Richards Bay area had the most evidence of endocrine disruption. This could be due to the aluminium smelters located at the Richards Bay harbour.

1.05.T-02 How Endocrine Disruptors May Affect Thymus Plasticity in Seasonally Spawning Fish

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Thymus and associated T cell-maturation are evolutionary conserved elements of adaptive immunity. The thymus is a highly plastic organ, the size and function of which changes in relation to reproductive functions. In mammals, puberty accelerates thymic involution with the rise of plasmatic gonadal hormones. In fish, thymic plasticity is also governed by environmental parameters such as temperature, photoperiod and food availability. During winter-season, the European sea bass, *Dicentrarchus labrax*, experiences lowered temperatures and reduced food intake. At the same time reproductive investment increases as the

fishes accomplish gametogenesis. We, therefore, analysed thymuses of juvenile fish by monthly samplings from 95 to 460 days post-hatching (dph), covering the first seasonal investment into gonad development. Animals were kept either in constant conditions, or in conditions mimicking natural variation of photoperiod and temperature. Temperature turned out as a key parameter that regulates the number of thymocytes. Gonadal development impacted both, the size of the thymus and thymic T cell-maturation, as increasing gonadosomatic indices reduced thymocyte number, their viability and the proportion of T cells. In constant conditions, however the number of thymocytes exponentially increased with the body weight up to 310 dph. We could further show that oestrogen modulates thymus development and thymus plasticity. Our results suggest that seasonal spawners regulate thymic plasticity and T cell-maturation *via* sex steroid hormones thereby attenuating adaptive immunity in order to adapt to winter-season and gametogenesis. As a matter of consequence, oestrogenic endocrine disrupting chemicals can interfere with this seasonal alternation of immune and reproductive function.

1.05.T-03 Species-Specific Activation of Human and Zebrafish Nuclear Receptors

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The spread of endocrine disrupting chemicals (EDCs) in the environment has recently raised concern about human and wildlife health. The molecular initiating event (MIE) of an adverse outcome pathway (AOP) induced by an EDC can be detected using *in vitro* cell lines expressing nuclear receptors (NRs), known to be targets of EDCs. While cell lines expressing human NRs have been widely used for the screening of suspected EDCs, cell lines expressing NRs of other species are less recurrent, leading the ecotoxicological evaluation of EDCs to extrapolate mammalian data to other species and raising doubts about their reliability. In this regard, we have established different cell lines expressing human (h) or zebrafish (zf) estrogen (ER), androgen (AR), progesterin (PR), glucocorticoid (GR) and mineralocorticoid (MR) receptors to investigate interspecies differences. Our results on ER expressing cell lines showed that endogenous, environmental, and synthetic ER ligands can induce transcriptional activity of ER subtypes with differences in selectivity and sensitivity. We also reported that many steroids had agonistic activity on hPR while being antagonist of zfPR. As regard the effects of chemicals on GR, few tested steroids showed differences in activation as were agonist on hGR and antagonist on zfGR. Moreover, on the hMR and zfMR, h and zfAR we are performing a screening of a bank of environmental chemicals, including pesticides, industrial compounds and pharmaceuticals in order to reveal if these known targets of man-made compounds present any relevant interspecies differences. Overall, we will identify the h and zfNRs for which interspecies differences in their activation by steroids and environmental chemicals exists. Due to the huge number of chemicals that need to be monitored and regulated, an integrated risk assessment which includes an in-depth analysis of the existing literature, *in silico* and *in vitro* data is needed to minimize animal testing as far as possible and assess at the same time the harmful effects of EDCs on human and wildlife health. To improve the EDC risk assessment, we therefore propose the integration of gene reporter assays expressing zfNRs in the evaluation of EDCs in aquatic toxicology.

1.05.T-04 Transcriptomic Responses to Sexual Endocrine Active Substances in Zebrafish Embryo

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Active substances of biocides or pesticides as well industrial chemicals can trigger adverse effects in environmental organisms with a far-reaching impact on ecosystems. To avoid such harmful effects, chemical manufacturers are required to provide data for environmental hazard and risk assessment of these chemical substances prior to their registration. Endocrine disruption - interference with the hormone system of the organism - is of particular concern because it can have long-lasting effects on populations even at relatively low substance concentrations. However, standardized assays for evaluating the impact of endocrine active substances on reproduction are expensive, in terms of both resources and animal use. Therefore, there is a need for alternative, cost-effective approaches, using animal replacement methods, with better predictive power to support hazard assessment. Omics-methodologies are attractive for collecting consistent high content data to discern chemicals modes of action (MoA) for hazard and risk assessment prioritization. To this end, we applied transcriptome profiling to a modified fish embryo toxicity test to analyze and compare estrogen and androgen-mediated endocrine disruption fingerprints induced by ethinylestradiol, tamoxifen, methyltestosterone and flutamide. Upon the 96 hours exposure, we did not observed any statistically significant mortality, neither in the control nor in the treatment groups. Although no significant phenotypic changes were observed following zFET endpoints, results from transcriptomic analysis revealed a concentration-dependent increase in the number of differentially regulated genes. Functional classification and overrepresentation analysis of the observed DEGs identified response to hormone, steroid metabolic process and cellular response to estrogen stimulus to be significantly perturbed. Our study demonstrates that omics-methodologies can significantly improve the identification of endocrine substances targeting reproductive physiological processes. The identified transcriptome fingerprints and in particular the identified signatures can be utilized for assessing sexual-related impairment of endocrine active agents in zebrafish embryos. Future screening approaches developed on the basis of such data will enable for an *ab initio* development of greener substances with less adverse effects on the aquatic environment.

1.05.T-05 Bisphenol a Alters Eye Morphology and Visually Mediated Behavior in Zebrafish (Danio rerio) Larvae and Juveniles Via Thyroid Hormone System Disruption

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The endocrine disruptor bisphenol A (BPA) is well known for its anti-estrogenic effect in vertebrates. In addition, it has been shown to disrupt amphibian metamorphosis, which is regulated by the thyroid hormone system (THS). Yet, the THS is not only initiating the metamorphic transformation from tadpoles to adult frogs but plays an essential role in the development of all vertebrates. In zebrafish larvae, THS disrupting chemicals have been shown to impair swim bladder inflation and interfere with eye development. The present study assessed the effects of BPA on the THS and eye development in zebrafish larvae and juveniles. Its aim was to further the understanding of how BPA affects the THS in developing zebrafish and to identify apical effects caused by this interference. To this end, zebrafish eggs were exposed to BPA for either seven (larvae study) or 63 days and histological assessments of the eyes and thyroid follicles, as well as behavioral studies, were performed. Larval whole-body thyroid hormone levels are currently being analyzed and will be presented. The results of the histopathology showed that eye morphology, specifically the thickness of distinct retinal layers, was significantly altered in larvae and juveniles. Furthermore, increased thickness of the thyroid follicular epithelium was observed in juveniles, indicating a compensatory response of the thyroid hormone system to a disruption caused by BPA. In behavioral studies with zebrafish larvae, a decreased swimming activity was observed in BPA-exposed individuals in response to changing light conditions (green, red, and white light, darkness). Taken together these findings suggest that BPA disrupts the THS in zebrafish thereby altering eye morphology and visually mediated behavior. This work provides further evidence that eye morphology is a suitable endpoint for assessing THS disrupting effects in both zebrafish larvae and juveniles.

1.05 Investigating endocrine-disrupting properties of chemicals: developments and challenges towards new approach methodologies (NAMs) (Part II)

1.05.T-06 Molecular Fingerprint of Thyroid Hormone System Disruption in Zebrafish (*Danio rerio*) and Its Reversibility **Rikke Poulsen¹, Pauline Pannetier², Lisa Gölz², Thomas Braunbeck², Lisa Annie Baumann³ and Martin Metabolomics Hansen⁴,** (1)Environmental Science, Aarhus Universitet, Denmark, (2)University of Heidelberg, Germany, (3)University of Heidelberg, Heidelberg, Germany, (4)Aarhus University, Denmark

Early vertebrate development is partly regulated by thyroid hormones (THs) and can therefore easily be disrupted by environmental pollutants that interact with the TH system (thyroid hormone system disrupting chemicals, THSDCs). It remains a question what the molecular fingerprint of this disruption is and whether effects are reversible. In a previous study, we show that continuous THSDC exposure of zebrafish embryos (*Danio rerio*) to two model THSDCs: propylthiouracil (PTU), a TH synthesis inhibitor, and tetrabromobisphenol A (TBBPA), which interacts with TH receptors, impacts the transcriptome as well as eye development and that the effects are partly reversible after a short recovery period of 3 days. In the current study we applied untargeted metabolomics and targeted analysis of 13 THs to look at the molecular signature accompanying the physiological events. Zebrafish embryos were exposed until 5 days post fertilization (dpf) to either PTU or TBBPA. Subsequently, half of the embryos were moved to clean water, to check for reversibility of effects, while the other half remained exposed until 8 dpf. Whole-body samples (n=20 animals per replica, N = 3 replicates), and dissected eyes and tails (n=40, N=3) to detect tissue-specific effects, were collected at 5 dpf and 8 dpf and homogenized for extraction of THs and metabolites. Preliminary analyses of the metabolome showed that >21 000 metabolite features are detectable in the samples, which can be grouped into 6923 compounds. Multivariate analysis of the data currently obtained confirmed the trend observed in the physiological endpoints: The treatment with the THSDCs caused changes in the metabolome compared to controls, however after the 3-day recovery period the metabolite pattern has been largely readjusted to control levels. In the targeted analysis of THs, it was possible to quantify 8 of the THs and several of these were affected by the treatments. In the recovery treatments compensatory responses and adaptation were observed, hence the morphologic, physiologic and transcriptomic effects were mirrored in the metabolome and further identification and annotation of metabolites will soon reveal the exact molecular fingerprint of the two model THSDCs in zebrafish embryos

1.05.T-07 The Impact of Thyroid Hormone System-Disrupting Chemicals on Zebrafish (*Danio rerio*) Eye Development **Lisa Golz¹, Lucia Vergauwen², Pauline Pannetier³, Dries Knapen², Henrik Holbech⁴, Thomas Braunbeck³ and Lisa Annie Baumann⁵,** (1)COS University of Heidelberg, Germany, (2)University of Antwerp, Belgium, (3)University of Heidelberg, Germany, (4)University of Southern Denmark, Denmark, (5)University of Heidelberg, Heidelberg, Germany

Thyroid hormones (THs) serve multiple functions; among others, THs regulate neurosensory, specifically eye, development in vertebrates. Also in fish, eye development is known to be directly regulated by THs and can therefore be used as thyroid-related endpoint in endocrine disruptor testing with fish. To investigate this, a set of THSDCs with different modes of action was selected to examine their impact on eye development after exposure during different life-stages of zebrafish (*Danio rerio*). Propylthiouracil, perchlorate, iopanoic acid and triiodothyronine were first tested for their toxicity in order to avoid interference with lethal concentrations in further testing of endocrine effects. Changes in thyroid follicle morphology of transgenic zebrafish embryos with fluorescent thyroid follicles were assessed to confirm specific impact on the TH system. Subsequently, exposure experiments were performed (1) until 5 days post fertilization (dpf) according to OECD test guideline (TG) 236, (2) until 28 days post hatch (dph) according to TG 210 or (3) until 60 dph according to TG 234. Effects on eye development were assessed by means of classic histology as well as immunohistology of different photoreceptors. For the development of an adverse outcome pathway (AOP) for disrupted eye development by THSDCs in fish, a detailed literature review was performed covering approximately 80 studies into the effects of TH level modification on eye development of fish. Based on the literature analyses and our experimental test battery with different model THSDCs, there is strong evidence that eye development and swim bladder inflation combined with TH level measurements and analyses of thyroid follicle morphology (histology or transgenic lines) could

be validated for implementation as thyroid-sensitive endpoints into existing TGs for endocrine disruptor testing with fish. Fundingby: This project has received funding from the European Union’s Horizon 2020 research and innovation program, under grant agreement no. 825753 (ERGO) and European Commission contract no. 07.0203/2018/794670/ETU/ENV.B.2 (“Development of a study protocol for regulatory testing to identify endocrine disrupting substances in biotic systems”).

1.05.T-08 Thyroid Hormone System Disruption Is LIFE Stage Specific in Fish: The CASE of Swim Bladder Inflation in Zebrafish

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Thyroid hormone system disruption (THSD) negatively affects multiple developmental processes and organs. Recently, an adverse outcome pathway (AOP) network was described, linking THSD to impaired swim bladder inflation and reduced swimming performance in fish. The swim bladder consists of two different chambers, an anterior and posterior, which inflate at different timepoints during development. During early embryonic development, inhibition of thyroid hormone (TH) activation (the conversion of thyroxine to triiodothyronine by deiodinase (DIO) enzymes) leads to impaired inflation of the posterior chamber. Inflation of the anterior chamber in juveniles is affected not only by inhibition of TH activation, but by inhibition of TH synthesis as well. In the present study, we examined the underlying mechanism through which THSD affects the posterior swim bladder by characterizing the timeframe during which the bladder is sensitive to DIO inhibition. Zebrafish embryos were exposed to iopanoic acid, a DIO inhibitor, during specific time windows. These time windows were chosen based on the three phases of posterior chamber development in zebrafish: the budding, pre-inflation and inflation phase. The budding and pre-inflation phase mark the formation of the swim bladder and the three structural tissue layers. During the inflation phase, the embryos gulp air to inflate the swim bladder. Impaired swim bladder inflation was observed in embryos exposed during the budding and pre-inflation phase, but not in embryos exposed during the inflation phase. These results indicate that the development of the bladder is sensitive to THSD, whereas the inflation process itself is not. To further elucidate the mechanism through which THSD affects the posterior swim bladder, additional analyses will be performed. These include gene transcription analysis, where both genes important in swim bladder development and inflation will be assessed, and analysis of TH levels. Knowledge gained in this research is not only relevant for the swim bladder but for other adverse effects of THSD as well. It is difficult to identify endpoints that are specific to THSD. Building a more comprehensive understanding of how THSD affects the different endpoints might allow us to identify more specific (combinations of) THSD biomarkers. These may then aid in further development of THSD screening methods. The contents of this abstract neither constitute, nor necessarily reflect, US EPA policy.

1.05.T-09 Consolidating the Interpretation of the Xenopus Eleuthero-Embryonic Thyroid Assay (XETA)

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The Organization for Economic Cooperation and Development (OECD) has set up a conceptual framework for the testing and assessment of endocrine disruptors. Different *in vitro* and *in vivo* standardized methods (test guidelines, TG) are categorized into five levels of increasing biological complexity. Detection of thyroid active molecules is addressed by mammalian and amphibian testing. Amphibian testing takes advantage of the THs’ exclusive control of anuran metamorphosis. The Amphibian Metamorphosis Assay (AMA) detects the actions of thyroid active molecules via physiological endpoints relevant for metamorphosis. In addition, in June 2019, the OECD validated the *Xenopus* Eleutheroembryonic Thyroid Assay (XETA) in the test guideline program. The XETA utilizes eleutheroembryos to detect modulation of thyroid signalling by thyroid active chemicals. The assay is transcription-based and uses a transgenic line expressing the Green Fluorescent Protein (GFP) under the control of a promoter directly regulated by TH. The response measured is fluorescence of embryos. When transcription of the genomic construct is activated or inhibited following chemical exposure, the embryos express more or less GFP and therefore emit more or less fluorescence compared to unexposed embryos. The XETA TG has been validated through an international effort via the OECD. The test of a set of 14 reference thyroid active chemicals with various known MoA (Mode of Action) demonstrated the transferability, reliability and reproducibility of the XETA but also highlighted the need for more data to consolidate the interpretation of the XETA with regards to the MoA that can be detected by this assay. In the present study, we selected 17 additional chemicals for which AMAs were available. In total, 31 reference chemicals have now been tested in the XETA. Information on the MoA is available for each test chemical. Overall, studies in line with the AMA are now available for a subset of 23 chemicals, allowing a comparison between the outcome of XETA and AMA. The results show that the XETA and AMA provide highly consistent results for different thyroid-related MoA, except NIS inhibition. This situation opens the possibility to use the AMA or the XETA or the two assays sequentially for regulatory assessment of chemicals or to screen for thyroid activity during the development of new chemicals.

1.05.T-10 Evaluation of an Adverse Outcome Pathway Network for Thyroid Hormone System Disruption Across Taxonomic Groups

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Thyroid hormone system disrupting chemicals (THSDCs) are widely regarded as potential threats to human and environmental health. Thus, efforts are underway within both the human health and ecotoxicology communities to develop screening assays capable of identifying THSDCs and to describe adverse outcome pathways (AOPs) that link thyroid hormone system disruption

(THSD) to adverse outcomes. When combining all AOPs for THSD in fish, mammals and amphibians, a cross-species AOP network for THSD emerges. While historically, human toxicological assessments of chemical safety have relied on rodent assays and ecotoxicological assessments have relied on non-mammalian assays including fish and amphibians, this cross-species AOP network provides an opportunity to break down this wall and explore avenues for integration of data from assays using different vertebrate taxa. AOPs describing THSD were retrieved from the AOP-Wiki (aopwiki.org). By filtering the resulting AOP network based on the currently described taxonomic domain of applicability of the AOPs (i.e., fish, amphibians or mammals), it was found that AOPs have been developed with a focus on one specific taxonomic group. While mammalian AOPs largely focus on developmental toxicity (DNT) as the adverse outcome, fish and amphibian AOPs thus far mostly lead to impaired swim bladder inflation and altered amphibian metamorphosis, respectively, both taxon-specific adverse outcomes. Other adverse outcomes include thyroid carcinoma, kidney toxicity, altered visual function, hearing loss and reduced fertility. Based on this evaluation, we identified data gaps and prioritized AOP development efforts. AOPs leading to altered visual function have high potential for cross-species AOP development. Since developmental neurotoxicity (DNT) is often considered the most important outcome of THSD in humans, and no AOPs for DNT in fish and amphibians exist, DNT was highlighted as an additional priority for cross-species AOP development and a potential route to expedite the use of fish and amphibian assays for predicting effects in humans. Next to DNT, there are other adverse health effects with potential for cross-species extrapolation including reduced fertility, hearing loss or neurosensory development in general, and kidney toxicity. Finally, another important gap is the lack of AOPs describing THSD in birds and reptiles. *The contents of this abstract neither constitute, nor necessarily reflect, US EPA policy.*

1.05 Investigating endocrine-disrupting properties of chemicals: developments and challenges towards new approach methodologies (NAMs) (Part III)

1.05.T-11 The Rapid Estrogen ACTivity In Vivo (REACTIV) Assay - Results From the OECD Validation Exercise

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Estrogen axis activity is currently being evaluated for a large number of chemicals, including obligatory testing for plant protection products under the Biocidal Products Regulation in Europe. Similar projects are also ongoing in Asia and North America. This has led to ethical and logistical problems which could be greatly reduced if whole animal, physiologically relevant, embryonic fish models, not compliant with the European definition of a laboratory animal, were available for medium throughput screening and prioritisation of chemicals. We developed an assay based on the use of transgenic medaka fish eleutheroembryos harbouring the *choriogenin h* promoter driving expression of *gfp* (*chgh-gfp*), the Rapid Estrogen ACTivity In Vivo (REACTIV) assay. The structural role of choriogenin proteins in egg formation suggests that this biomarker may, like the highly related vitellogenin biomarker, not only reveal estrogen axis activity, but may also accurately predict impaired reproduction. In addition to the lead laboratory in France, partner laboratories in the UK, Japan, Denmark, the USA and Germany have been recruited for the interlaboratory validation exercise. The test chemicals chosen for this exercise cover a wide range of mechanisms of action. The REACTIV assay is performed by exposing newly hatched fry of the *chgh-gfp* line to the test chemical in the presence and absence of testosterone (30 µg/L). This allows quantification of the effects of chemicals acting via mechanisms of action revealed in the presence of an aromatisable androgen (e.g. aromatase inhibition, estrogen receptor antagonism) as well as those more easily detected in the absence of estrogen axis signalling such as estrogen receptor agonists. Exposure studies are carried out in six-well plates for 24 h with one test group of eleuthero-embryos per well. The ongoing OECD validation exercise has so far produced some encouraging results. The transferability of the assay as well as its ability to identify chemicals acting on estrogen axis signalling, either at the receptor level or on downstream steroidogenesis and its selectivity have been demonstrated. The eleutheroembryonic life stages on which the assay is based, fall outside of the EU definition of a laboratory animal, in line with the 3R's principle of animal replacement. This assay is currently being evaluated by the OECD as a level 3 screening assay.

1.05.T-12 Optimising Concentration Setting for In Vivo Endocrine Disrupting Chemical Identification Studies

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There is growing regulatory demand for fish and amphibian *in vivo* data to support identification of chemical-induced endocrine activity and disruption. To ensure that only endocrine-specific effects are detected and aid data interpretation, improvements to concentration-setting guidance are needed. This will help avoid triggering unnecessary further vertebrate tests and the use of concentrations that cause unnecessary suffering in test animals. Determining the most appropriate highest concentration – or maximum tolerated concentration (MTC) - for these studies is problematic, e.g. there is inconsistency in how this is defined and recommended within different Organisation for Economic Co-operation and Development (OECD) test guidelines. This work aims to provide a consensus definition of the MTC for fish and amphibian endocrine tests and develop an optimised strategy for concentration setting. Preliminary analysis of regulatory fish (rainbow trout) acute toxicity data on 22 substances assessed the appropriateness of setting the MTC using 1/3rd of the acute LC₅₀ value or 1/10th of the acute LC₅₀ value. These values were compared to the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) to evaluate if (sub)lethal symptoms of toxicity were observed in proximity to the selected MTCs. Comparing acute outcomes with the MTC derived from 1/3rd LC₅₀, in 5/22 (23%) of cases the MTC would not be appropriate as it exceeded the LOEC for (sub)lethal effects. In 7/22 cases (32%) the MTC may not be appropriate as it fell between the NOEC and LOEC values for sublethal effects. Comparing acute outcomes with the MTC derived from 1/10th LC₅₀, in 1/22 (5%) of cases the MTC would not be appropriate as it exceeded the LOEC for sublethal effects. In 4/22 (18%) of cases the MTC may not be appropriate as it fell between the NOEC

and LOEC values for sublethal effects. These results highlight the potential for confounded outcomes in fish endocrine tests depending on how the MTC is selected. Next steps include: 1) conducting the same comparisons for fathead minnow data; 2) conducting the same analysis for amphibians; and 3) comparing different approaches for MTC setting with endocrine-relevant endpoints in fish short term reproduction and amphibian metamorphosis assays to determine a) whether overt toxicity was observed in the test concentration range, and b) if this range was sufficient to allow for detection of endocrine activity.

1.05.T-13 The Population Relevance of Endocrine-Mediated Adversity for Non-Target Organisms: Challenges and Future Needs

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In June 2018, a guidance document for the implementation of the ED criteria has been published. Similar to the ED criteria, the guidance is based on the definition of WHO/ICPS of an endocrine disruptor: a substance should be considered an ED if it causes 'A change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or population, in the case of non-target organisms, that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences'. The criteria also stipulate that, in applying the WoE approach, the assessment of the scientific evidence shall consider the adverse effects on reproduction, growth/development, and other relevant adverse effects which are likely to impact populations of non-target organisms. Thus, when defining adversity for non-target organisms the relevance of the observed effects in laboratory studies for the maintenance of the population needs to be addressed. This means that different levels of extrapolation are needed: from laboratory to field conditions and from individual to population. It is known that populations of non-target organisms in the natural environment are able to compensate the effects observed in laboratory to a certain extent and some ED related effects are only transitory. However, from a regulatory perspective, the population relevance of the effects observed in laboratory studies is assumed by default, with few exceptions, unless further proven. The aim of the present work is to present: I. the overview of the challenges identified following the implementation of the ED criteria and the application of the ECHA/EFSA guidance with regard to the population relevance of ED-mediated adversity on non-target organisms (e.g. vertebrates); II. the gaps identified for the determination of the population relevance of the adversity and the future research needs. Based on the experience gained with the evaluations done so far for pesticides, further research is needed on: (i) the extrapolation from lab to realistic field conditions; (ii) the pattern of effects for each taxon which may lead to population impairment; (iii) the use of population models for addressing the population relevance including consideration on e.g. the species to model.

1.05.T-14 New Approach Methodologies (NAMs) for the Endocrine Activity Toolbox: Environmental Assessment for Fish and Amphibians

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Evaluation of endocrine activity in human and wildlife involves specific *in vivo* and *in vitro* assays used to evaluate relevant pathways. Multiple *in vivo* OECD test guidelines have been validated for mammals, amphibians, or fish, focusing on the estrogen, androgen, thyroid, and steroidogenesis (EATS) pathways. However, these toxicity tests often require the use of a large number of laboratory animals, which are cost inefficient and contradict the 3R principles for animal welfare considerations and increasing mandates to move towards an "animal free" testing paradigm worldwide. New approach methodologies (NAMs) hold great promise to identify molecular changes that can be used to predict individual or population effects and to reduce the numbers of animals used in ecotoxicology testing. In a collaborative effort led by HESI and the UK's NC3Rs, experts from government, academia, and industry met in 2020 to discuss the current challenges of endocrine toxicity testing for fish and amphibians. In continuing the cross-sector initiative, follow-up work is focusing on the current state of the science to evaluate chemical endocrine disruption potential in amphibians and fish using NAMs to predict effects for chemicals acting via EATS pathways. This work highlights the challenges of using NAMs for risk or hazard assessment and what is needed to reduce the uncertainties with the use of these tests. Movement towards development and utilization of NAMs not only allow for replacement, reduction, and refinement of animal testing but also provide robust and fit for purpose methods to identify chemicals acting via endocrine mechanisms. We have summarized current NAM methods available for endocrine activity assessment including *in silico*, cell-free & cell-based methods, and embryo models. This effort focuses on endocrine activity evaluation for fish and amphibians, but conservation of many key endocrine pathways across species allows approaches used in other contexts (e.g., human health assessment) to be leveraged. In addition, the use of pathway-based approaches to inform testing applies to non-endocrine mechanisms. These NAMs can be integrated into Adverse Outcome Pathway (AOP) frameworks within a weight of evidence (WoE) approach for hazard or risk assessment. The application and use of the NAMs within an IATA or WoE approach will be described within specific problem formulation contexts – protection goal, data needs, and testing requirements.

1.05 Investigating endocrine-disrupting properties of chemicals: developments and challenges towards new approach methodologies (NAMs) (Poster)

1.05.P-Mo033 In Vitro Evaluation of Potential Estrogenic Compounds on Estrogen Receptors of European Sea Bass (*Dicentrarchus labrax* L.)

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Estrogens are important regulators of many physiological processes such as reproduction and immunity and estrogenic action is mainly mediated by hormone binding to estrogen receptors. Specific transcription factors designated nuclear estrogen receptors (Esrs) have been associated with the activation of gene expression by estrogens or structurally similar compounds. Three subtypes of Esrs have been identified in teleosts. Recently, membrane G protein-coupled estrogen receptors (Gpers) have been related to rapid non-genomic responses. Both signaling pathways are found in fish which may express additional gene duplicates for both receptor types. Estrogen-like endocrine disrupting chemicals (EEDCs) are known to have an impact on the environment at large. Aquatic species are particularly concerned as their environment is the final receptacle of several contaminants. Various changes in physiological functions, such as reproduction, development or immunity are well described in the scientific literature. In this context, the identification of EEDCs at the source appears to be of a major interest. OECD *in vitro* assays to screen EEDCs already exist, but they only consider two human Esrs which are not necessarily representative of Esrs found in teleosts. Recently, the importance in teleost of Esr2b as a significant signaling pathway, yet ignored by existing screening tests, was emphasized. Therefore, we propose a more holistic *in vitro* test using gene reporter assay that includes sea bass Esr1, Esr2a, Esr2b, Gpera and Gperb. Transient transfections using transactivation of an ERE or CRE-luciferase reporter assay for the nuclear and membrane receptors, respectively, were performed on HEK293 cell line and validated using 17 β -estradiol (E2). Using this test, we aim at evaluating the estrogenic potency of putative EEDCs included in the European Agency for Chemical Compounds or the French Agency for Food, Environmental and Occupational Health & Safety list: dexmedetomidine, galaxolide, homosalate, thiram, triphenyl phosphate and triclocarban. They are tested singly and in mixture with E2. By considering Esrs and Gpers, risk assessment of EEDCs could be more accurate allowing to understand their mode of action via various signaling pathways. Its routine use should facilitate the identification of problematic compounds, their elimination at source and the protection of environmental health. This work was funded by the Interreg France (Channel) England program (RedPol project).

1.05.P-Mo034 Interaction of the Thyroid Hormone System With Eye Development in Fish: An Adverse Outcome Pathway Approach

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To a significant extent, early vertebrate development is regulated by thyroid hormones (THs) and can, therefore, readily be disrupted by environmental pollutants which interact with the TH system (thyroid hormone system-disrupting chemicals, THSDCs). THs serve multiple functions, among others, neuromorphological, specifically eye development in vertebrates: Also in fish, eye development is known to be directly regulated by THs and can, thus, be used as a thyroid-related endpoint in endocrine disruptor testing with fish. However, the interaction between THSDCs and eye development in fish has not yet been sufficiently explored. Especially with regard to ecological consequences of impaired eye development, it is essential to understand the full sequence of events from molecular interaction with the TH system over morphological and physiological changes up to adverse outcomes at individual and population levels. For development of an Adverse Outcome Pathway (AOP) for disrupted eye development by TDCs in fish, a detailed literature review was performed covering approximately 120 studies into the effects of modification of TH levels on eye development in fish. The studies reviewed include a variety of different approaches for detection of TH disruption, including THSDCs, transgenic or mutant fish, microinjection, morpholino oligonucleotides, thyroid ablation, etc.. Endpoints range not only from gene and hormone level modification to cellular changes in the eyes (e.g. size of cells, cell layer structure, organization and amount of photoreceptors, pigmentation, etc.), morphological changes (e.g., eye size or shape), but also include physiological and behavioral changes (e.g. optokinetic response, light response, swimming activity). The literature analysis provides clear evidence that different modes of THSDCs result in impaired eye development in fish. Results confirm the growing evidence that fish eye development is sensitive to THSDC treatment and might represent a promising endpoint for the assessment of thyroid-related effects in fish, maybe being indicative for eye-related effects in vertebrates in general. The AOP will build the basis for further research on the consequences of TH system disruption in fish (vertebrates), yet, a research field still with multiple knowledge gaps. Funding by the European Union's Horizon 2020 research and innovation program, under grant agreement no. 825753 (ERGO).

1.05.P-Mo035 Morphological Changes in the Retina and Thyroid Follicles of Zebrafish (*Danio rerio*) Embryos: CASE Study With Resorcinol

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Resorcinol is commonly used as a medicinal ingredient in human dermatology, in rubber industries (about 50% of total use) and the production of high-quality wood glues (about 25% of adhesive markets). Moreover, it has been reported to act as a thyroid hormone system disruptor. Alterations of thyroid hormonal pathways can lead to a variety of adverse effects, because of the crucial role of the thyroid in various metabolic, behavioral and developmental processes. Effectively, inhibition of deiodinase enzymes, which catalyze thyroxine (T4) deiodination to triiodothyronine (T3), can lead to profound disturbances in the regulation of thyroid hormones. To detect endocrine disrupting compounds with adverse effect on thyroid-related processes, alternative tests using zebrafish (*Danio rerio*) embryos have been developed. Thyroid-sensitive endpoints within these tests were swimbladder inflation, eye development, thyroid follicle morphology/histopathology and thyroid hormone levels. The present study examined resorcinol effects on eye development and thyroid follicle morphology following 5 days exposure to up to 100 mg/L resorcinol on early life-

stages of the zebrafish embryos. Compared to the water control, resorcinol reduced the thickness of the retinal pigment epithelium (RPE) as well as the inner and outer segments of the outer nuclear layer (ONL). Ocular malformations and reduced thickness in the internal plexiform layer (IPL) were also noticed. Moreover, the thyroid follicle size showed a significant increase, which confirmed the specific impact on the thyroid hormone system of zebrafish.

1.05.P-Mo036 Targeted vs. Non-Target Identification of Endocrine Disruptors by Gene Expression Analyses As an Additional Ecotoxicological Endpoint in Fish Studies: Challenges and Opportunities

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When investigating potentially endocrine-disrupting substances using existing test guidelines, it is important to evaluate rapid-response indicative parameters in addition to population-relevant endpoints. They can provide important information on the underlying mechanisms of action. A promising approach is the investigation of molecular endpoints, as they react very quickly to pollutants. This approach was tested in the course of two Zebrafish Extended One Generation Reproduction Tests, which were supplemented at different life stages by qPCR expression analyses of genes identified to be sensitive for the substance-specific modes-of-action. The study detected gene expression changes at different life stages, which fit comparably well to the expected changes for a substance acting as aromatase inhibitor, while the results for an estrogen receptor modulator were less predictable. It was observed that the expression of selected genes highly depends on the life stage and the chosen tissue; for example, expression changes in head tissue might be of less relevance for sex-specific genes; this observation is supported by the literature. However, the approach revealed some limitations, as a targeted analysis of pre-selected genes may hinder the identification of endocrine active substances with unknown MoA. More comprehensive approaches, such as transcriptomics (e.g. by RNAseq), would allow the identification of MoA-specific expression patterns as molecular fingerprints, serving as data base for substances with unknown endocrine activity. Those non-target analyses of gene expression further allow the identification of MoA-specific gene expression biomarkers, which could be more easily combined with existing test guidelines. For application, a tiered approach should be considered. Tier 1 will be represented by the identification of gene expression biomarkers by global gene expression analyses (e.g. transcriptomics) during a screening approach with embryonic fish. Within a second tier, i.e. chronic fish studies like the FELLS test will be conducted coupled with an integrated qPCR analyses of biomarkers identified in the first tier. This approach would not only allow the establishment of a causal link between the substance's MoA and the adverse effects, which is prerequisite for the identification of endocrine disruptors, but would also meet the 3R requirements for animal welfare, as no additional animal studies would be required for a substantial gain of knowledge.

1.05.P-Mo037 Effects of Thyroid Hormone Axis Disrupting Chemicals on *Rana temporaria* Metamorphosis

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Thyroid Hormone Axis Disrupting Chemicals (THADCs) interfere with the functioning of the thyroid hormone axis, resulting in detrimental effects on critical biological functions like brain development in vertebrates. Amphibians are particularly sensitive to THADCs, as thyroid hormones play a critical role in metamorphosis. OECD test guidelines used to investigate the effects of THADCs have been conducted on *Xenopus laevis* and *Xenopus tropicalis*, tropical frog species with a 100% aquatic life cycle, which may not be representative of European semi-aquatic amphibian species. We collected *Rana temporaria* eggs in Spring for three consecutive years, to investigate the effects of three THADCs on the common frog metamorphosis and thyroid histology, using a shortened Larval Amphibian Growth and Development Assay (LAGDA). *Rana* eggs were exposed to 11.9 - 426.5 µg/L sodium perchlorate (SP, 2018), 1.23 - 47.7 mg/L 6-Propylthiouracil (PTU, 2019), and 18.59 to 349.62 µg/L tetrabromobisphenol A (TBBPA, 2020) for around 6 weeks. At the end of the exposure, developmental stage, morphological data (i.e., total length, body weight, and hindlimb length) and thyroid histopathology (i.e., thyroid gland size, number and size of the follicles, follicle cell height) were assessed. We observed that PTU strongly delayed development without affecting growth, thus resulting in younger, bigger tadpoles. PTU also affected thyroid histopathology, inducing a strong increase in thyroid gland size from the lowest concentration, and follicles with more irregular shapes and proportionately less lumen and colloid. TBBPA only slightly decreased total length and increase the size of follicles' lumens, and no effect was observed for SP. All in all, our results showed that thyroid histopathology endpoints are more sensitive than developmental stages and morphological data, and *R. temporaria* may be more sensitive to PTU but less sensitive to SP than described in the literature for *Xenopus*.

1.05.P-Mo038 Reversibility of Thyroid Hormone System-Disrupting Effects on Eye Development of Zebrafish (*Danio rerio*)

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Early vertebrate development is partly regulated by thyroid hormones (THs) and can, therefore, easily be disrupted by environmental pollutants which interact with the TH system (thyroid hormone system-disrupting chemicals, THSDCs). It is known that the eye development of fish is directly regulated by THs and can, therefore, be used as thyroid-related endpoint in endocrine disruptor testing with fish. We were interested to see whether THSDC-induced malformations of eyes during early development would be reversible after depuration in clean water. To this end, zebrafish (*Danio rerio*) embryos were exposed until 5 days post-fertilization (dpf) to either propylthiouracil (PTU), a TH synthesis inhibitor, or tetrabromobisphenol A (TBBPA), which interacts with TH receptors. Subsequently, 50 % of the embryos were exposed further to the THSDCs until 8 dpf, and 50 %

were kept in clean water to check for reversibility of effects. Continuous THSDC exposure altered eye size and pigmentation together with changes in the cellular structure of the retina. Interestingly, the effects were partly reversible after the short recovery period of 3 days. The results are in line with changes in TH levels measured in different tissues of the embryos, for example locally in the eyes. Results show that eye development in zebrafish embryos is highly sensitive to THSDC treatment, but eyes are apparently able to recover rapidly from early exposure *via* effective repair mechanisms. **Funding:** This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 825753 (ERGO).

1.05.P-Mo039 Implementation of Thyroid-Sensitive Endpoints Into the Fish Early Life Stage Test (FELS, OECD TG 210) With Zebrafish (*Danio rerio*) - Part 1: Thyroid Follicle and Gonad Histopathology

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Currently, endocrine disruptor (ED) testing is conducted with multiple protocols using various species both for human health and environmental safety evaluation. ERGO, as part of the EU H2020 EURION cluster project, is focused on the optimisation of such existing test guidelines (TGs) for screening for ED effects of chemicals. Modified or new TGs are expected to both comply with the reduction, replacement, and refinement (3R) principle in animal testing and to reduce the time and costs needed for the evaluation and authorisation of new and existing compounds. The Hypothalamus-Pituitary-Thyroid (HPT) axis as a crucial component of homeostasis and developmental processes is preserved across vertebrate classes. Except for thyroid hormone system disruption (THSD), zebrafish (*Danio rerio*) has become a well-established model in ED testing. In order to optimise current testing procedures, the addition of new thyroid-related endpoints to existing TGs with for zebrafish is (over)due. From mammalian and amphibian studies, it is known that the morphology of the thyroid follicular epithelium is directly affected by thyroid hormone system-disrupting chemicals (THSDCs) as a response to interference with TH homeostasis. Consequently, histopathological analyses of thyroid follicles appear mandatory also in tests with fish. To check for the sensitivity of thyroid histopathology in fish, two model compounds with known THSD properties, iopanoic acid (IOP) and potassium perchlorate (PCL), were used to identify effects on thyroid follicle morphology in a fish early-life stage toxicity test (FELS, OECD TG 210). In order to further increase the ecological relevance, an additional recovery phase of 30 d was added after the initial 34 d exposure period. Furthermore, a histopathological analysis of the gonads is currently being carried out to assess crosstalk with the HPG axis. With regard to the target organ, the thyroid, PCL and IOP exposure had no statistically significant effects on thyroid follicle morphology, when measured at 34 dpf. After 64 d however, statistically significant differences in thyroid epithelium thickness were observed in exposure and recovery groups for both substances. The results show that implementation of thyroid epithelium thickness into TG 210 is technically feasible. However, refinement is needed to, e.g., replace amphibian testing. Moreover, life-stage sensitivity and general developmental effects must be considered when analysing THSD in TG 210.

1.05.P-Mo040 Implementation of Thyroid-Sensitive Endpoints Into the Fish Early Life-Stage Test (FELS, OECD TG 210) With Zebrafish (*Danio rerio*) - Part 2: Eye Development and Thyroid Hormones

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Currently, both for human health and environmental safety evaluation, endocrine disruptor (ED) testing requires multiple studies across different species. The EU H2020 project "ERGO" is focused on the optimisation of existing test guidelines (TGs) relevant to endocrine activity and adversity for human and environmental health. New TGs would be beneficial to the 3R (replace, reduce, refine) principles and would also reduce time and costs for ED testing. The Hypothalamus-Pituitary-Thyroid axis (HPT) is highly conserved and plays important roles in both early development and homeostasis across vertebrate classes. Except for thyroid hormone system disruption (THSD), zebrafish (*Danio rerio*) is already commonly used as a model species in ED assessment. In order to expand existing TGs to allow for a comprehensive assessment using a single species only, new thyroid-related endpoints have to be evaluated in different developmental stages. Given that eye development is directly regulated by thyroid hormones (THs), possible endpoints include eye malformations. For this purpose, a fish early life-stage toxicity test (FELS, OECD TG 210) with iopanoic acid (IOP) and potassium perchlorate (PCL) was conducted. These substances were selected for their known effects on the HPT axis with different modes of action. In addition to the 34-d exposure phase, a recovery phase of 30 d was added to determine the potential to recover from adverse effects on eye development. We assessed the eye-to-body-ratio, the thickness of the inner plexiform layer (IPL), the retinal pigment epithelium (RPE) and the photoreceptor layer (PL) as well as the ratio of the outer segment (OS) of the PRL to the total PRL thickness. These endpoints have previously been shown to be sensitive to disruption of the HPT in zebrafish embryos. Results show that eye malformations also manifest in later life-stages but tend to be reversible after recovery in clean water. Significant differences were observed for the RPE and the IPL at both time points with the OS/PRL ratio showing significant effects at 64 d. These results support the inclusion of the eye development, especially the thickness of the RPE, as a thyroid-sensitive endpoint into TG 210, thus allowing for a comprehensive assessment of ED effects in a single species. This would help reduce the number of amphibian studies required. TH level measurements are ongoing and will provide mechanistic data for our understanding of effects observed at apical level.

1.05.P-Mo041 A New Standard for Endocrine Disruptor Testing in Fish - the Integrated Fish Endocrine Disruptor Test (iFEDT)

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Various recent EU research projects deal with the optimization of aquatic vertebrate-based test systems for the identification of endocrine disruptors (EDs). Attempts are made to cover all relevant life-stages, to identify EDs with different modes of action, to partly replace existing amphibian tests and to include thyroid-related endpoints in existing fish test guidelines (TGs). For this end, the EU tender project “Integrated Fish Endocrine Disruptor Test (iFEDT)” was designed to establish a new test system that combines and extends two existing fish test guidelines: the Fish Short-Term Reproduction Assay (OECD TG 229) and the Fish Sexual Development Test (OECD TG 234). Propylthiouracil (PTU; 0 - 78 mg/L; n = 4) and ethinylestradiol (EE2; 0 - 10 ng/L; n = 4) were tested as model substances for disruption of the thyroid hormone and the sexual hormone systems, respectively. In addition to endpoints established in the existing OECD TGs (fecundity, growth, gonad histopathology, vitellogenin, etc.), various thyroid-related endpoints such as hormone levels, thyroid histopathology and (eye) development were assessed during the 85-days exposure period. Results from the first experiment indicate that PTU not only impairs the reproductive performance of adult fish and reduces the growth of juvenile fish, but also induces strong thyroid follicle proliferation in all life stages. Moreover, histopathology of the eyes demonstrates that this endpoint is very sensitive to thyroid disruption in younger life-stages. The experiment with EE2 confirms a strong impact on reproduction of parental fish, on gonad development in the offspring and on vitellogenin levels in either life-stage. Based on these observations, the merged test design iFEDT appears to be a promising tool to integrate various important ED modalities in fish into one assay covering all sensitive life-stages. Funding: This study receives funding from the European Commission under contract no. 07.0203/2018/794670/ETU/ENV.B.2 (“Development of a study protocol for regulatory testing to identify endocrine disrupting substances in biotic systems”).

1.05.P-Mo042 New Transgenic Medaka Model to Detect Disruption of Thyroid Signalling

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Endocrine disruption caused by chemicals has been considered as a major issue over the last few decades for both human and wildlife health. Identifying endocrine disrupting chemicals in order to limit their usage is therefore a priority. EU authorities has defined a testing strategy to determine the potential endocrine activity on EATS (Estrogen, Androgen, Thyroid, Steroidogenesis) axis. Regarding thyroid disruption, three OECD guidelines using *in vivo* non-mammalian models are validated, all of them are based on amphibian metamorphosis. In the most recent assay, the XETA (Xenopus Eleutheroembryo Thyroid Assay, OECD N°248), an amphibian (*Xenopus laevis*) transgenic line expresses a fluorescent protein which is quantifiable and proportional to thyroid axis activity. To date no OECD test guideline based on fish are available for the detection of thyroid active chemicals. We aim to fill this gap by developing a new test for the detection of thyroid active chemicals using medaka eleutheroembryos (*Oryzias latipes*). This test involves a transgenic line, expressing Green Fluorescent Protein (GFP) under the control of the *thyroglobulin* promoter (a precursor of thyroid hormones). We first identify the medaka sequences presumably involved in the regulation of the expression of *thyroglobulin*. A transgene was then built by cloning this putative promoter upstream of the *gfp* coding sequence. Series of micro-injections were performed in one-cell embryos in order to develop transgenic lines. Screening the offspring reveals that five transgenic lines were obtained with different transmission rates but all having fluorescent thyroid follicles. We are now studying the five transgenic lines to characterize the spatio-temporal expression of the fluorescence and the modulation of fluorescence intensity by pro and anti-thyroid reference chemicals. Variation of fluorescence intensity is expected to reflect the negative feedback loop of HPT-axis. Using this fish test along with amphibian assays will allow a comparative physiology strategy offering a way to understand specific thyroid regulating mechanisms that are not fully elucidated in fish. Developing a test using transgenic medaka offers the possibility to combine this assay with the RADAR and/or REACTIV assays that are currently in OECD validation. This strategy could lead to reduce the time, number of eleutheroembryos and cost associated with EATS assessment in fish.

1.05.P-Mo043 Toward an AOP Network-Based Testing Strategy for the Assessment of Thyroid Hormone System Disruption in Fish

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Thyroid hormone system disruption (THSD) has been recognized as an import focus area in the context of hazard and risk assessment of chemicals worldwide where further development of assays and endpoints is needed. In particular for fish-based test guidelines (TGs), no endpoints for THSD have been validated yet. Various research projects are currently ongoing or being planned to address this challenge, including the H2020 ERGO project, the EU DG Environment funded iFEDT project, the US EPA Chemical Safety for Sustainability Program and the Horizon Europe PARC partnership. These activities are focused on increasing our fundamental understanding of TSHD, reviewing existing knowledge, and developing a fish TSDH AOP network to support the selection of assays for inclusion in TGs. From the perspective of fish test method development it is especially important to systematically document the high degree of life stage specificity of the different THSD mechanisms and effects in fish. This level of resolution in the domain of applicability description is required to determine which endpoints are relevant in the context of non-protected life stage tests such as the Fish Embryo Acute Toxicity test (OECD TG 236), and which endpoints need to be assessed in juvenile or adult tests. A number of endpoints are currently well documented, including swim bladder inflation, eye development, thyroid follicle histology and thyroid hormone levels. Other endpoints under consideration include fin development, skin pigmentation and gene transcription in target organs. It is likely that the level to which these individual endpoints are specific to THSD may differ and it is therefore anticipated that a combination of endpoints may be required. The envisioned overall implementation strategy is to add endpoints that are supported by OECD-endorsed AOPs to existing fish TGs

by establishing a direct connection between relevant projects of the OECD's AOP development work plan and the work plan for the TGs programme. Specifically, an AOP network for THSD in fish is being developed in project 1.35 of the AOP development programme and a first set of 5 AOPs is currently being considered for EAGMST approval and endorsement by WNT/WPHA. This work will be integrated with project 2.64 of the work plan for the TGs programme, which aims to include thyroid endpoints in existing OECD fish TGs. In this context, a pre-validation effort of a selection of relevant endpoints is expected to start in 2022.

1.05.P-Mo044 Are Hormone Measurements the Best Way to Look for Endocrine Disruption?

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Endocrine disrupting chemicals pose a threat to both humans and wildlife. The hypothalamic-pituitary-thyroid axis has been identified as an area with large knowledge gaps and insufficient test guidelines, not at least when it comes to early life stages. In this study we used targeted quantification of 13 thyroid hormones as well as an untargeted metabolomics to investigate the molecular fingerprint in mouse plasma following *in utero* and lactational exposure to thyroid hormone system disrupting compounds (THSDC). We tested two chemicals, propylthiouracil (PTU) and tetrabromobisphenol-A (TBBPA), that are known to disrupt the thyroid hormone system but have different molecular initiating events for their toxicity. Pregnant C57Bl/6 mice were exposed to either PTU (1 mg/L in drinking water) or TBBPA (by gavage, 2 mg/kg/day dissolved in oil) from gestational day 7 to postnatal day 14. Pups were then sacrificed and their blood sampled for analysis on a nano-liquid chromatography (LC)-Orbitrap system. Statistical analysis showed no significant effects of the THSDCs on the thyroid hormones. However, metabolomics analysis revealed 85 up- and 152 down-concentrated metabolites due to PTU exposure and 134 up- and 69 down-concentrated metabolites due to TBBPA exposure ($p < 0.05$, fold change > 1.5). Annotation revealed that many of the affected metabolites were involved in fatty acid metabolism incl. linoleic- and alpha linoleic acid metabolism. Hence the study allowed identification of metabolites that might be used as more sensitive key events to assess the thyroid hormone system disrupting potential of putative THSDCs and identify other affected biochemical pathways

1.05.P-Mo045 Comparison of NF Developmental Stage-Matched Control Data in Amphibian Metamorphosis Assay Continuous Quantitative Endpoints (HLL, SVL, WET WEIGHT)

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The analysis of continuous quantitative *Xenopus laevis* endpoints such as snout-to-vent length (SVL), hind limb length (HLL), nHLL (normalized by SVL) and wet weight is performed to assess statistically significant differences between the negative control and active substance treatments in the Amphibian Metamorphosis Assay (AMA) for the detection of potential thyroid modes of action according to Guidelines OECD 231 and OPPTS 890.1100. However, the current statistical analysis of these parameters at Day 7 and 21 is routinely performed by using the replicate means or medians of the recorded HLL, nHLL, SVL and wet weight individual values without taking into consideration the different stages of development within each control or treatment replicate. Furthermore, these endpoints are well known to be directly impacted by developmental stage in tadpoles and so using stage-matched data may have an impact in the interpretation of the biological results. This is also the case for tadpole thyroid gland histopathological findings, where it is already recognised in both Guidelines that the most appropriate approach is to use NF developmental stage-matched tadpoles for evaluation. Consequently, individual control HLL, nHLL, SVL and wet weight values on Day 7 and 21 from the available data set have been extracted and evaluated according to NF developmental stage. The analysis presented here aims to develop a NF developmental stage-matched Historical Control Database (HCD) that allows the assessment of potential impact in the outcome of statistically significant results of these thyroid screening parameters, as well as the variability of NF developmental stage-matched control data across different GLP AMA-performing laboratories.

1.05.P-Mo046 Challenges With the New XETA Test System

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The OECD Test Guideline No. 248 (Xenopus Eleutheroembryo Thyroid Assay, XETA) was published in June 2019. Since then, several laboratories have started establishing the test at their facilities. In this poster presentation, the major challenges of the establishment of the test at a new facility are shown. According to the guideline, the establishment phase includes testing four proficiency chemicals at each new facility in order to demonstrate that the desired results can be reached. These reference tests show the high variability within this test and indicate the limitations of this test. One additional challenge discussed will be that not all laws, even within Europe, are equal. The test was developed as an alternative to animal trials and the EFSA recognizes it as such. This means the embryos are not recognized as vertebrates up to the stage where the test ends according to the EU regulations. This is, however, different in Switzerland, where the animals are seen as vertebrates at this stage. Thus, the test is not considered as an alternative to animal tests, which defeats the purpose of replacing the fish tests by the XETA. Further challenges are substance related. Various examples will be shown of substances that cannot be tested with the standard XETA set-up and how these challenges can be overcome. This includes substances that have a very short half-life under test conditions, substances that adhere to plastic, and substances that are degraded by the biological material within the test. The possibility of running this test in a flow-through system is also explored.

1.05.P-Mo047 Retrofitting In Vitro Test Systems for High Throughput Screening of Thyroid Disrupting Chemicals With Metabolic Capacities - a Tiered In Vitro/In Silico Testing Strategy

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Many compounds unfold their toxic potential only after bioactivation, as also encountered for endocrine disruptors. Nevertheless, commonly used HTS approaches for endocrine disruptors do not include xenobiotic metabolism, even though most in vitro test systems do not preserve the metabolic activity of their parent tissue of origin. Retrofitting in vitro test systems with metabolic capacities is a promising strategy to solve the stated issue. However, current approaches hamper HTS applications due to costs and technological practicality. On the contrary, cellular tests systems augmented with external metabolism capacities (liver S9 fractions) seem adjustable to an HTS scenario, as proven for ER and AR (ant-)agonistic effects, if parameters are appropriately adjusted. Here, we report the conceptualization and application of an HTS in vitro/in silico testing battery to assess thyroid disrupting potential. Two HepG2-based reporter cell systems (TH metabolism; TH signalling), an enzymatic turnover assay (TH synthesis), and a TH transporter assay were assessed regarding their compatibility with S9 external metabolization incubations parallel to chemical exposure. The parameters for potential cytotoxicity and cofactor concentrations were optimized. Additionally, in silico biotransformation models (Biotransformer, QSAR toolbox, CTS) are employed to derive consensus metabolites, which are assessed for a potential de- or increase in toxicity via TR a/b and TTR-inhibition QSARs. The obtained in silico data will qualitatively be compared to in vitro results within a tiered testing approach. The depicted HTS testing scenario complies with devised OECD short and midterm strategies on endocrine disruptors. Indeed, S9 liver fractions are animal-derived components that need to be phased out shortly. However, liver S9 fractions currently serve as a consistent regulatory-accepted biotransformation tool within in vitro toxicology until biotechnological alternatives have matured. The project has received funding from the EU H2020 project ERGO (No.825753).

1.05.P-Mo048 Multiple Mechanisms of Action of Pollutants and Their Mixtures in Aquatic Environment Associated With Thyroid Hormone Disruption

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While endocrine disruption associated with steroid hormones is a well-described phenomenon nowadays, there is less info related to interference of chemicals with the thyroid hormone (TH) system. Nevertheless, TH plays a crucial role in many vital processes such as metabolic activity, neurodevelopment, swim bladder inflation in fish or amphibian metamorphosis. Xenobiotics that can interfere with the thyroid hormone (TH) system (Thyroid hormone Disrupting Chemicals; TDCs) have been linked with numerous disorders across different species. This poses an urgent need for the development and implementation of suitable methods for the screening of chemicals as well as environmental pollutant mixtures for their TH-disrupting properties. Unlike in steroid hormone systems, receptor-mediated effects are probably not the most important mode of action of TDCs. TH synthesis, transport, and metabolization steps in TH metabolism are likely more important targets for TDCs. We have developed a set of bioassays focusing on prioritized endpoints in thyroid hormone disruption. The assays for the assessment of effect of TDCs on iodide uptake by thyroid cells mediated by Na⁺/I⁻ symporter (NIS) are based on stably transfected human cell line overexpressing NIS and on rat thyroid cell model, and on the detection of uptaken iodide levels by the cells using a non-radioactive, colorimetric Sandell-Kolthoff reaction. Transport of TH is addressed by fluorometric assessment of displacement of TH from its plasma transporter transthyretin (TTR) by TDCs. The TH metabolization step is addressed by a cell model with a reporter gene under transcription control by aryl hydrocarbon receptor (AhR) that plays a significant role in triggering the metabolization systems. Interaction with thyroid receptor signaling is assessed using reporter-gene assay. These assays have been established and optimized for screening of a set of prioritized chemicals and environmental samples from the aquatic environment for their potential to disrupt the thyroid hormone system. The results demonstrate the utility of the newly developed bioassays for high-throughput screening of chemicals as well as environmentally relevant complex pollutant mixtures for the characterization of their thyroid hormone-disrupting potential.

1.05.P-Mo049 Findings on Sexual Development of Zebrafish Exposed to Parabens - Does Metabolization Play a Crucial Role in the Context of Endocrine Disruption?

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Parabens, the alkyl esters of p-hydroxybenzoic acid, are widely used as preservatives in consumer products, primarily in cosmetics and pharmaceuticals. Their toxicological profile is well characterized, and they are suspected of having endocrine disrupting (ED) properties. This suspicion is based primarily on studies of estrogen agonistic activity in vitro conducted according to Level 2 of the OECD ED Conceptual Framework. To assess the biological relevance of estrogen receptor bioactivity of parabens, parameters correlating with ED-relevant endpoints from fish studies on propyl- and methylparaben are discussed. Based on REACH requirements Fish Sexual Development Tests (OECD TG 234) on methylparaben and propylparaben had to be conducted. The performed in vivo studies were required by ECHA during the REACH registration process. The evaluation of the ED related endpoints revealed no adverse effects on parameters such as male to female ratio, increase of vitellogenin in male fish, or other population relevant endpoints for methylparaben and propylparaben. Based on the results of both studies, there is no evidence that methyl- or propylparaben has ED properties leading to an adverse effect. One explanation for this observation could be that both parabens are rapidly metabolized in zebrafish and the metabolites have less or no endocrine activity. Consequently, in a second study, the metabolic capabilities in different life stages of the zebrafish towards parabens were investigated. For this

purpose, two different concentrations of methyl- and propylparaben were tested in each case upon 96 h exposed eggs, 14 days exposed juvenile fish and 63 days exposed adult fish. One key aspect was whether early developmental stages exhibit different metabolization rates than older developmental stages. The identification of the metabolites of methylparaben and propylparaben were performed using liquid chromatography high resolution mass spectrometry (LC-HR-MS) and fragmentation experiments (MS/MS). Seven metabolites of methylparaben and eight metabolites of propylparaben were identified. First findings show high capability to metabolize parabens at all 3 life stages of zebrafish. This could be an explanation for the fact that no evidence of an ED related adverse effect was observed.

1.05.P-Mo050 Using the ProtoCol 3 Colony Counter to Quantify Egg Numbers From the Fish, Short Term Reproduction Test

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The OECD 229 Test Guideline requires daily assessment of reproduction as one of the measurements of test item effect for potential endocrine disruption with a daily desirable egg production at least 10 eggs per female per day in the control. For a valid test this equates to a minimum of 3360 eggs from the control group alone; however, experience has shown that with fathead minnows, egg production can be considerably higher than this with 40-60 eggs per female per day a routine occurrence. To compound this, a typical test requires a minimum of 80 females per test therefore often double this number of fish are usually used for monitoring egg numbers during the pre-acclimatisation stages. Given these higher number of eggs it was decided that a manual counting procedure would extremely time consuming and potentially inaccurate and therefore an automated approach was adopted. A ProtoCol 3 Colony Counter was selected which was able to considerably speed up the counting process with quantification taking less than 2 minutes for each test vessel once the eggs had been collected and stained. On a daily basis, the breeding tiles containing fathead minnow eggs were removed from the test vessels. Once the eggs have released from the tiles, they were stained, sieved and rinsed and placed in a petri dish to be quantified using the Protocol 3 Colony Counter. Some validation studies were conducted to ensure the suitability of the counter: Three different sized (small, medium and large) broods of eggs were collected and prepared for counting using the Rose Bengal stain. Each batch of eggs was counted five times using the ProtoCol 3 Colony and Counter and once each by five different technicians. The length of time taken to manually count the eggs was also recorded. Here we present the results of our validations. The major benefit is the significant reduction in time taken to count the eggs.

1.05.P-Mo051 A Critical Review on Assessing Anti-Androgenic Activity of Chemicals in Fish

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According to the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA) endocrine disrupter (ED) guidance (ECHA/EFSA 2018), to sufficiently investigate the oestrogenic, androgenic and steroidogenesis modalities, a Fish Short-Term Reproduction Assay (FSTRA, OECD TG 229), or 21-day fish assay (OECD TG 230) are preferred. However, concerns have been raised by EFSA and in the OECD GD 150 that such assays may not be sufficiently sensitive to detect anti-androgenic effects of chemicals. This view has led to requests for additional testing, such as the Fish Sexual Development Test (FSDT; OECD TG 234), despite the lack of evidence that such a test would be more sensitive. Therefore, we have conducted a review of *in vivo* studies to assess the sensitivity of fish and the ED relevant endpoints to anti-androgenic substances. Work to date has included: · Use of the United States Environmental Protection Agency's (US EPA's) Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) database to investigate the conservation of the Androgen Receptor (AR) between vertebrates and fish species. Findings show that the AR is well conserved at all model levels and in all OECD fish model species (e.g. *Pimephales promelas*, *Danio rerio*, *Oryzias latipes*, *Gasterosteus aculeatus*). · ToxCast™ anti-androgenicity scores were used to 15 model anti-androgenic substances for inclusion in the review. · A literature search of the prioritised model substances was conducted which identified over 50 fish *in vivo* studies with endpoints which would be included in an ED assessment. These results have shown that the sensitivity of endpoints to the anti-androgen modality was inconsistent within, and across, the various fish studies when exposed to the most potent anti-androgenic substances. Neither the FSTRA nor the FDST were more sensitive in detecting this endocrine modality. The poster will present the outcomes of the work to date, and evidence on the suitability of fish study designs and endpoints, to detect the anti-androgenic modality.

1.05.P-Mo052 The Interplay of Paraoxonase 1 and Bisphenol a in Metabolic Disruption

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Endocrine disrupting chemicals (EDCs) have long been studied for their effects on steroid hormone pathways. Recent studies have shown that some EDCs also have a metabolic disrupting effect, which could result in fat accumulation in the liver when lipid metabolism is disturbed. However, the details of these interactions are still not fully understood. One such interaction is between EDCs and paraoxonase 1 (PON1), an enzyme with antioxidant functions, known to be associated with fatty liver and to metabolize xenobiotic substances. In order to better understand the mechanism by which PON1 is affected by metabolic disruption via dietary changes or metabolic disrupting chemicals, experiments with zebrafish were performed under different metabolic conditions. First, diet quantity was changed in order to simulate metabolic disruption. Secondly, metabolic disruption was induced by feeding with a custom obesogenic diet in combination with exposure to different doses (5 µg/kg, 50 µg/kg and 500 µg/kg) of a metabolic disruptor, bisphenol A (BPA). During each trial, the weight and length of each fish were followed up.

After 6 weeks, PON1 activities, oxidative stress, BPA concentration, lipid profiles, energy reserves and mRNA expression of genes involved in energy metabolism or downstream effects of bisphenol A are measured in liver tissue to characterize the relations between these parameters. Changes in diet quantity resulted in a difference in condition factors. These changes were correlated negatively with pon1 lactonase activity, which in turn correlates positively with oxidative stress. This resulted in a few hypotheses: 1) This is an attempt to use the antioxidant function of PON1 to reduce oxidative stress, 2) PON1 activity is increased by metabolic signaling pathways, or 3) it is a combination of both. However, the change in the condition factor induced by an obesogenic diet did not result in a different pon1 lactonase activity. Instead, exposure to BPA resulted in an increase in lactonase activity for the highest concentrations. Oxidative stress will be measured in the same samples to test its relation to the changes in lactonase activity. These changes will be compared to internal BPA concentration, changes in lipid profiles, and mRNA expression of relevant genes to obtain more mechanistic insights.

1.05.P-Mo053 Bridging the Gap Between Drug Exposure and Sex-Related Gene Expression in Environmental Toxicology Studies

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Gender has previously been shown to affect the way individuals respond to toxicological exposure. Genes which allow identification of sex in juvenile zebrafish show potential to aid confounding variables in environmental toxicology trials but the link between these is so far missing. Early expressed gender specific genes where expression is not altered by drug exposure must be carefully and specifically selected for this purpose. Transcriptome data from Next-Generation-Sequencing (NGS) of adult male, adult female and juvenile *Danio rerio* were used to discover sex-specific juvenile-expressed genes. Previous studies were used to classify juvenile-expressed genes to highlight the interference of these juvenile biomarkers with toxicological application to uncover gender-related variations in gene expression during environmental toxicology trials. Four male and eight female genes (*Gapdhs*, *Apobb1*, *Spata6* and *Dmrt1* and *Bmp15*, *Chr4*, *Zpb3*, *Gyg1a*, *Kpna2*, *Ftz-f1*, *gdf9* and *Ipo4*, respectively) show great potential for use in revealing sex related differences during environmental toxicology studies. Discovery of these early sex-determining genes will allow identification of gender-related responses to toxicity testing to improve sex-specific healthcare and the medical treatment of patients.

1.05.P-Mo054 Comparison of Internal Historical Data From Endocrine Disruptor (ED) Screening Assays With Data From the EPA ED Screening Programme

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Testing for endocrine disruption is an essential part of the regulation of chemical substances in the EU and USA. Key screening assays are the Fish Short Term Reproduction Assay (FSTRA), OECD 229 (OCSP 890.1350), the 21-day Fish Assay (FA), OECD 230, and the Amphibian Metamorphosis Assay (AMA), OECD 231 (OCSP 890.1100). In particular for the registration of plant protection products (PPPs) in Europe these assays are critical, since the indication of an effect triggers higher tier tests resulting in additional costs and additional use of animals and, in case of a confirmed endocrine disrupting effect, a ban of the substance on the European market. Since 2019 IES Ltd has been conducting FSTRA, FA and AMA on a regular basis using fathead minnow (*Pimephales promelas*) and the African clawed frog (*Xenopus laevis*). During implementation and performance of these assays we identified gaps in the description of the applied test performance in both the OECD and the OPPTS (OCSP) guidelines, which may affect the reproducibility and comparability of the outcomes. Given the high importance of these tests for the registration and re-registration of PPPs, existing gaps should be discussed and closed soon. To identify weaknesses of the current procedures, we compared our results for all relevant endpoints (fecundity, nuptial tubercle score, and vitellogenin for the fish tests and determination of the Nieuwkoop-Faber stage, measurement of the hind-limb and snout-vent length for the *Xenopus* test) with data from the Endocrine Disruptor Screening Program, published by the EPA. The results of this comparison will be presented and critically discussed. Moreover, we will provide recommendations to improve the testing procedure, such as the choice of suitable test concentrations.

1.05.P-Mo055 Locomotor Activity of Zebrafish Larvae After Exposure to Plastic Additives

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Plastic additives are added to plastic to improve the functional properties necessary for its end use or application. They are not chemically bound to the plastic and when not properly disposed of, eventually leach out in the environment. These compounds are suspected endocrine disruptors capable of altering the behaviour of affected animals. This study aimed to evaluate the behavioural toxicity of environmentally-relevant concentrations of di-2-ethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP) on zebrafish larvae. Day 0 Zebrafish embryos were exposed to (0,0.5, 5, 50, 500) µg/l and (0, 0.5, 5, 50) µg/l concentration of DEHP and DBP respectively for 96 hours. The survival rate and developmental abnormalities were observed while the locomotive activity was automatically tracked using Zantiks MWP_vb unit. Startle protocol was used to induce movement for 22 mins and the percentage of animals moved during the startle stimulus (light on/off or vibration) was recorded. In animals exposed to DEHP, no mortality was observed, while DBP had 100% mortality in 50 and 500 µg/l after 96 hours. This study found a significant effect of DEHP on startling response in both baseline and vibration conditions. Larvae in 5 µg/l moved slower than those in control and the rest of the exposed group, showing a U-shaped response. The percentage of larvae moved in the baseline, and vibration stimulus had a significant concentration-response effect after exposure to DBP. In conclusion, the tested plastic additives reduced the overall

activity of zebrafish larvae, although the effects were more evident in animals exposed to DEBP than DBP. This suggests that the introduction of vibration as an additional stimulus influenced their startle response. The mode of action of these compounds may cause differences in how they affect the survival and locomotor activity of zebrafish.

1.05.P-Mo056 Comprehensive Analysis of the Literature to Construct AOP Network Related to Metabolism Disorders Induced by Endocrine Disruptors Using the Aop-Helpfinder Tool

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Adverse outcome pathways (AOPs) are a conceptual framework that support the use of alternative toxicological approaches in chemical risk assessment. AOPs provide structures organizations of known biological perturbations, starting from an initial molecular event (MIE) to an adverse outcome (AO) across several intermediate key events (KEs). To help the development of AOPs and AONs (AOP networks), which are a combination of several AOPs sharing at least one biological event, we have developed a tool named AOP-helpFinder. AOP-helpFinder is based on artificial intelligence, more precisely using natural language processing (NLP) and graph theory to systematically and rapidly explore all available abstracts stored in the PubMed database. It will identify and extract known associations between stressors of interest and KEs, therefore supporting the development of AOPs. Recently a web-server has been created to facilitate the use of this advanced bioinformatics tool. The web server operates with an updated version of the tool, that allows to refine (using machine learning, i.e. lemmatization for text normalization) and reduce (capability to search only in the results/conclusion part of an abstract) the searches. In the context of the H2020 OBERON project, we investigated the applicability of using the AOP-helpFinder tool with an integrated systems toxicology approach to develop an AON related to metabolic disorders, that are among the main adverse health outcome associated with endocrine disrupting chemicals (EDCs). Co-occurring terms (i.e. EDCs and biological events (MIE, KE, AO) from the AOP-wiki database and from in house experts) were identified by the AOP-helpFinder tool. This step allowed to decipher known links between EDCs and biological events, and was followed by a manual curation to select the most relevant publications. Then, AON was proposed, which was enriched by integration of complementary data extracted from other databases (e.g. CompTox). Computational approaches that allow identification, extraction and integration of existing knowledge, appear to be essential to have a better understanding of the mode of action of suspected EDCs, and the biological pathways that may perturb in order to identify their real impact in the human population.

1.05.P-Mo057 Perinatal Exposure to Permethrin and Bisphenol F Alters Lipid Metabolism in Rat Liver

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Human exposure to EDCs during pregnancy and early stages of life can disrupt normal patterns of brain development and reproductive function, leading to severe pathologies later in life. Environmental studies have linked bisphenols (BPA mostly) and phthalates exposure to neurodevelopmental diseases. However, a wide number of suspected EDCs such as pyrethroids, are hardly studied in relation to neurodevelopmental diseases. Lipidomics showed to be a powerful tool in environmental toxicology in recent years: the key function of lipids in brain homeostasis and liver metabolism makes them exceptional candidates in the discovery of sensitive biomarkers for neuroendocrine toxicity in animals and humans. The liver is the key organ for the maintenance of whole-body lipid homeostasis with the potential to influence brain function. The study investigated, therefore, the effects of two chemicals used worldwide, bisphenol F (BPF), a substitute of the EU-banned BPA, and the pyrethroid permethrin (PMT), on lipid metabolism. Female and male rats were exposed to BPF (0.036 mg/kg; 3.6mg/kg BW/day) or PMT (0.36 mg/kg BW/day; 3.6mg/kg BW/day): the dams were exposed during pre-mating, mating, pregnancy, and lactation; the pups were exposed until postnatal day 6 (PND 6) *via* maternal milk. Lipid profiles were determined in both exposed and control groups, focusing solely on PND 6 rat livers. Liquid chromatography combined with high-resolution mass spectrometry (LC-ESI QTOF) was used for lipidomics analysis. MS features extraction (m/z), signal intensity, RT, peak picking, and lipid MS/MS spectral matching were performed through MS-DIAL. Data normalization and statistical analysis as ANOVA (p-values < 0.05, FDR), PLS-DA and fold change analysis were performed with MetaboAnalyst. The Lipid Ontology (LION) pathway enrichment analysis was also investigated to link lipid disruption to specific cellular compartments in the liver. The results showed that many lipids were affected due to BPF or PMT exposure, and these effects were gender and dose-specific: BPF downregulated triacylglycerols, glycerolipids, and glycerophosphoethanolamines, and upregulated glycerophospholipids, glycerophosphocholines, and fatty acids. PMT upregulated diacylglycerols, diacylphosphocholines, and fatty acids, and downregulated glycerolipids, glycerophosphoglycerols, and triacylglycerols. The results showed that BPF and PMT have significant effects on the liver lipid homeostasis of rat offspring.

1.05.P-Mo058 An Adverse Outcome Pathway Linking Retinoid Signaling Disruption to Craniofacial Malformation and Feeding Behavior Impairment

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Retinoid acid (RA) is an essential morphogen during fish early development. Its anteroposterior gradient concentration is a key actor during embryonic patterning and morphogenesis. Although retinoid signaling disruption is understudied, recent studies

demonstrated the presence of environmental pollutants with retinoid-like activities. We have identified some gaps in the development of retinoid-associated adverse outcome pathway (AOP), whereas there is already extensive evidence linking the disruption of RA gradient and teratogenicity. In this study, we aim to develop an AOP linking retinoid signaling disruption to functional impairment in fish. Specifically, we propose an AOP linking the agonism of the heterodimer Retinoic Acid Receptor (RAR) / Retinoid X Receptor (RXR) with craniofacial malformation and feeding disruption in zebrafish early development. To trigger the molecular initiating event, we selected compounds activating RAR / RXR through different mechanisms in retinoid signaling. To unravel the temporal concordance and essentiality, we exposed zebrafish embryos during several time windows (0-24hpf; 0-48hpf; 0-72hpf; 0-96hpf) and compare craniofacial malformation at 120hpf with a) dose-response Meckel-Palatoquadrate (M-PQ) angle score and b) morphological chondrocyte assessment. After determining EC20, EC50, and EC80, we perform an ingestion test to explore functional adverse effects at the individual level. According to Dynamic Energy Budget (DEB) theory, the energy uptake is allocated to different physiological functions such as growth, maturation, maintenance, reproduction. A feeding behavior impairment would affect the energy uptake. Consequently, the normal physiological functions could be disrupted and lead to population decline. The project is a part of the PRORISK network, which aims to incorporate mechanistic, ecological, and socio-economic aspects into environmental risk assessment. PRORISK has received funding from European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 859891 (MSCA-ETN).

1.05.P-Mo059 Linking Chemical Information and Receptor-Mediated Effects - a CASE Study on 56 European Wastewater Treatment Plant (WWTP) Effluents

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Aquatic ecosystems are impacted by mixtures of endocrine disrupting chemicals (EDCs), including different steroids and phenols. These chemicals are released into the environment via wastewater treatment plant (WWTP) effluents, among other sources. Due to their high potencies at low environmental concentrations, they require adequately sensitive detection methods and assessment approaches. Scientists from the field of ecotoxicology have been reporting on the potential of linking information on the occurrence of chemicals (exposure) and adverse biological effects (toxicity) [1, 2]. In the present case study we used chemical and effect-based tools to screen 56 European WWTP effluents for various EDCs. The main objectives were (a) to investigate a combined approach of chemical target screening and receptor-based testing for estrogenicity, glucocorticogenic activity, androgenicity and progestogenic activity (ER⁺-, GR-, AR- and PR-GeneBLazer assay) in treated wastewater, (b) to compare three different receptor-based assays for estrogenicity testing (ER⁺-GeneBLazer, pYES, ER⁺-CALUX®), and (c) to evaluate the potential impact of advanced wastewater treatment technologies on both exposure and toxicity. As a result, steroids and phenols were detected at concentrations ranging from ng/L to µg/L. In the GeneBLazer assays, ER⁺- and GR-activity was detected in 82% and 73% of the samples, respectively, while AR- and PR-activity were only measured in 9% and 20% of the samples, respectively. 17-β estradiol and triamcinolone acetonide were the predominant drivers of estrogenicity and glucocorticogenic activity, respectively. The comparison of BEQchem and BEQbio for ER, GR, AR and PR demonstrated a good correlation of chemical target screening and receptor-based testing results ranging within an order of magnitude above or below ideal correlation. WWTP effluents which have passed an advanced treatment via ozonation and AC were found to be less contaminated, in terms of lower or no detection of steroids and phenols, as well as receptor-mediated activities. Results on estrogenicity from the ER⁺-GeneBLazer assay and the p-YES test showed stronger correlations between BEQchem and BEQbio than the results of the ER⁺-CALUX® assay. This study shows that future water quality monitoring and assessment of certain steroids and phenols can be improved by linking chemical and effect-based screening tools.

1.05.P-Mo060 Interrogation of the GPCRome by Food Contact Chemicals

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The adverse health effects from endocrine-disrupting chemicals (EDCs) in plastic products have been well documented over the past decades. It has become clear that exposure to food contact chemicals (FCCs), that is compounds migrating for food packaging, can interfere with the endocrine system to cause developmental or reproductive toxicity, increased risk to cancers, and disturbances in the immune system. Traditionally, EDC research has focused on nuclear receptors while largely ignoring their potential to interact with cell surface receptors. G-protein coupled receptors (GPCRs) are the largest family of cell surface receptors and are a primary point of research in drug discovery. Emerging research has shown that GPCRs are activated by PCBs, PAHs, pesticides, and other environmental contaminants. In this work, we have utilized the PRESTO-Tango (Parallel Receptorome Expression and Screening via Transcriptional Output, with transcriptional activation following arrestin translocation) to identify the potential of FCCs to act as agonists on a set of 135 previously validated human GPCRs. We show that well-known plastic chemicals, as well as the mixture of compounds present in food packaging, can activate a variety of GPCRs, including melatonin (MT1 and MT2), melanocortin (MC3R), and prostaglandin (PTGER1) cell surface receptors. This agonism has been further validated through secondary beta-arrestin dose-response curves. This work demonstrates that plastic chemicals can activate GPCRs, and, most importantly, highlights that cell-surface receptors are relevant, yet understudied mechanisms of action for

EDCs and other compounds which may be important to the increased incidence and severity of certain non-communicable diseases.

1.05 Investigating endocrine-disrupting properties of chemicals: developments and challenges towards new approach methodologies (NAMs) (Virtual Only)

1.05.V-01 Adult Zebrafish Exposures to a Progestin Mixture and Ethinylestradiol Lead to Gonadal Structural Changes

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Endocrine disruptors (EDCs) and, in particular, ubiquitous chemicals capable of mimicking natural oestrogens, known as xenoestrogens, have been a hot study area. However, there is a lack of research on the effects of oestrogen and progestin combinations. Over the years, experimental data have pointed to important concerns for species of many taxa, as well as considerable ecological effects. The purpose of this study was to determine the impact on the gonadal volume and gametogenesis kinetics of adult zebrafish exposed to a progestin mix and ethinylestradiol (EE2). Thirty-two zebrafish females and 32 males (in 4 groups) were exposed for 28 days to solvent (ethanol at 0.1%), progestins mix (100 ng/L of levonorgestrel and of megestrol acetate), progestins mix + EE2 (100 ng/L + 10 ng/L) and EE2 alone (10 ng/L). We studied the maturity grade (1 to 5), gonadal volume, and GSI values as a first step. The gonadal volume and GSI did not vary significantly among groups, but there was a marked trend towards lower mean and median values in females exposed to the mixtures and EE2 alone (especially in the latter group). In general, these results agree with comparable studies. In terms of ovarian maturity grading, the control group had a median score of 3, whereas the EE2 and progestin mix groups had less developed maturity scores (1.5). However, there were no significant differences. The progestin + EE2 mix group showed a smaller degree of ovary maturation, significantly differing from the control. Stereology was used to estimate the relative volumes (RV) of the ovarian compartments to refine the semi-quantitative approach. Relative to control (12%), the primary oocytes' RV was greater in all exposed groups (19%). Furthermore, females exposed to EE2 had decreased RV of advanced oocytes stages and increased atresia and stroma. Despite a decreasing trend, and in contrast with the grading, the RV of the mature oocyte compartment did not differ between the control (54%) and progestin + EE2 mix group (33%). The outcome indicates that the EDCs used had a direct influence on gametogenesis, as hypothesised, altering oocyte maturation. Overall, differential effects were found in females after exposure to EE2 and EE2 + progestins. This fact supports that co-exposure to progestins modified the impacts of EE2 alone. The study will be refined by analysing the total volumes of the ovary structures. Acknowledgements: Funded by FCT and ERDF (UIDB/04423/2020, UIDP/04423/2020) and ICBAS.

1.05.V-02 Identification of Thyroid Disruption Molecular Markers Based on Transcriptomic Analysis: Optimization of Protocol Exposure for RNA Sequencing Using Whole Zebrafish Embryos

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Nowadays, environmental pollution is rising concern for both human and environmental health. Among others, the evaluation of endocrine disruptor chemicals is a challenge for both regulators and industry. More specifically only a few methods exist to identify Thyroid disruptor compounds (TDC). Those methods are low throughput, and expensive. Moreover, [LM1] some such as the AMA (Amphibian Metamorphosis Assay) do not comply with the animal testing ban of cosmetic regulation. Therefore, it is becoming urgent to develop novel strategies and screening methods to identify TDCs. As thyroid signaling is highly conserved between teleost and mammals, zebrafish embryo is an ethical alternative model for studying both physiological regulations and disruption. Our main objective is to identify and validate robust biomarkers for Thyroid Disruption in the zebrafish, To do so, we design a transcriptomic analysis where embryos are exposed to reference compounds alone or in combination with thyroid hormones. We selected four reference compounds (Iopanoic acid (IOP), Sodium Perchlorate (PCL), Tetrabromobisphenol-A (TBBPA) and 6-propylthiouracil (PTU)) based on their different modes of action on the thyroid signaling pathway. Then we performed FET (Fish Embryo Test - OECD guideline, n°236) as a range-finding test of reference compounds to choose what concentrations fits better to RNA sequencing. Experimentally, a total of three biological replicates containing 40 whole zebrafish embryos each will be exposed, RNAs extracted and deep sequenced. Data will be processed and analyzed with in-house pipelines. Lists of differentially regulated genes (DEG) will be classified in several clusters, each corresponding to a type of biological response. This is an essential step as it makes the transition between raw data and biological results. In addition, biological responses will be modeled with the system biology framework, where DEG will be placed in their functional context by building a network of signaling and metabolic pathways. This set of data, integrated in the context of the H2020 European project ERGO will provide the molecular basis for the development of an adverse outcome pathway network as well as new endpoints and molecular biomarkers.

1.05.V-03 Identifying Potential Endocrine Active Substances on the Canadian Domestic Substances List Using Version 2.0 of the Ecological Risk Classification of Organic Substances Approach: A Case Study

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Environment and Climate Change Canada has recently finished development of version 2.0 of the ERC approach (ERC2) for approximately 12 200 organic substances from the Domestic Substances List (DSL) that did not meet criteria for further risk assessment following Canada's 2006 PBT-oriented categorization exercise. ERC2 is a high-throughput IATA method using many sources of 'alternative data' (new approach methodologies, or NAM) such as *in silico*, *in chemico*, and *in vitro* data to complement traditional *in vivo* sources to provide evidence for risk classification and other regulatory activities. A case study is

presented demonstrating the approach used in ERC2 to identify potentially endocrine active substances. Data were collected for estrogen (E) receptor, androgen (A) receptor, and thyroid (T) receptor interactions, as well as affinity for the aryl hydrocarbon receptor (AhR). Potency-based hazard classification rules were developed to classify ERC2 substances for the above receptor-mediated interactions for *in silico*, *in vitro* data and mammalian and aquatic *in vivo* data nodes. An Adverse Outcome Pathway structure associates the mechanistic data with the *in vivo* data to determine plausible interactions. Confidence scoring rules measure the degree of data availability and consensus among the data nodes using a weighted data preference hierarchy. Relatively few *in vivo* sub-lethal effects data were available for profiling receptor-mediated effects for the ~12 200 chemicals in ERC2, but a high percentage of *in vitro* data and *in silico* predictions were available or generated for ERC2 chemicals. Just under 18% of all ERC2 substances trigger rules for potential endocrine activity identified by the ERC2 receptor-mediated descriptor, regardless of confidence score. Approximately two-thirds of these have low or very low confidence scores, suggesting significant *in vivo* data gaps and lack of potency consensus between data types. Follow-up regulatory actions (e.g., no further action, data gathering, risk/hazard assessment) can be proposed for ECCC future regulatory activities based on the combination of hazard potency (i.e., hazard classification) and confidence score.

1.05.V-04 Investigation of Thyroid Endocrine Disruption of Two Isothiazolinones Using *Thraa*^{-/-} Zebrafish Embryo *Sujin Lee¹ and Kyunghye Ji², (1)researcher, Yongin, Korea, Republic of (South), (2)Yongin University, Korea, Republic of (South)*

Isothiazolinones are widely used as preservatives or biocides in various household chemical products. Toxicity studies have been conducted to investigate the adverse effects to skin sensitization and irritation; however, limited information is available on thyroid endocrine disruption. In the present study, we developed a novel *in vivo* thyroid endocrine disruptor screening system employing wild-type and thyroid hormone receptor alpha a knockout (*thr^a^{-/-}*) zebrafish embryo, and applied them to two isothiazolinones, i.e., benzisothiazolinone (BIT) and methylisothiazolinone (MIT). To test the validity of our *in vivo* thyroid endocrine disruptor screening system, we evaluated the sensitivity profile of *thr^a^{-/-}* to T3 and propylthiouracil. *Thr^a^{-/-}* zebrafish seemed to detect thyroid endocrine disruption with a significantly higher sensitivity than did the wild-type. Two types of zebrafish embryo were exposed to BIT and MIT (0, 0.03, 0.3, 3, 30, 300 µg/L) for 96 h, and compared the larvae mortality. *Thr^a^{-/-}* zebrafish exhibited a hypersensitivity than wild-type to BIT and MIT, suggesting the possibility of thyroid endocrine disruption based on inhibition to bind thyroid hormone receptor alpha. Acknowledgement: This study was supported by National Research Foundation of Korea (NRF; Project no. 2019R1A2C1002712).

1.05.V-05 Studying the Effects of a Xenoestrogen and Warmer Temperature on the Guppy Fish Liver Size and Cellularity *Margarida Vilaça¹, Maria Rocha¹, Sukanlaya Tantiwisawaruj² and Prof. Eduardo Rocha¹, (1)ICBAS and CIIMAR, University of Porto, Portugal, Portugal, (2)Learning Institute, King Mongkut's University of Technology Thonburi, Bangkok, Thailand, Thailand*

Pollutants, notably endocrine disruptors, have a negative impact on aquatic habitats and the organisms that inhabit them. When processed by the liver, these chemicals can exert an influence on the organ and produce morphological and functional alterations. Aside from that, the rise in water temperature caused by global warming has unavoidable effects on ectothermic creatures such as fish, including disruptions in metabolic processes, reproduction, growth, and survival rates. However, our understanding of the final combined impacts of endocrine-disrupting chemicals and temperature remains restricted. As a result, the purpose of this study is to see if a higher temperature and exposure to 17-ethinylestradiol (EE2) affect (independently or interactively) fish liver mass and cellularity. To put our hypothesis to the test, we performed a stereological investigation using light microscopy. We utilized 25-µm-thick paraffin sections of livers obtained from an experiment with mature male guppies (*Poecilia reticulata*). The animals were divided into four groups: two solvent control groups kept at 26°C or 29°C for 45 days (simulating warming), and two groups kept at the same temperatures but exposed to EE2 (at a nominal concentration of 50 ng/L). The findings revealed that EE2 and temperature influenced body biometry, with EE2 having a detrimental effect on body mass and length. According to preliminary stereological data, fish from the control groups exhibited larger livers. This fact was consistent with greater body biometry. Furthermore, control fish appear to have bigger or more hepatocytes (this is under analysis). The findings suggest that the higher temperature affected the core structure of the liver. At this stage, we can not tell if the temperature influenced the effects of EE2 on liver variables. Whatever the study's final conclusion, the new data will help to shed light on the still-unknown processes of fish liver reactions to both stimuli. Funding: Supported by FCT and ERDF (UIDB/04423/2020 and UIDP/04423/2020) and ICBAS.

1.06 Metagenomics tools to unravel environmental adverse effects on communities (Poster)

1.06.P-Tu001 RNA Metabarcoding to Reveal Responses of Zooplankton Community to Environmental Stressors *Phillip Ankle¹, Yuwei Xie², Sonya Havens³, Lisa Peters⁴, Jose Luis Rodriguez Gil⁵, Lauren Timlick⁴, John Giesy¹ and Vince Palace⁵, (1)University of Saskatchewan, Canada, (2)Toxicology Center, University of Saskatchewan, Saskatoon, SK, Canada, (3)IISD Experimental Lakes Area (IISD-ELA), Winnipeg, MB, Canada, (4)University of Manitoba, Canada, (5)IISD Experimental Lakes Area (IISD-ELA), Canada*

DNA metabarcoding can provide a rapid, high-throughput method for characterizing responses of communities to environmental stressors. However, within bulk samples, DNA metabarcoding has issues distinguishing live from dead organisms. Here, both DNA and RNA metabarcoding were applied and compared in experimental freshwater mesocosms conducted for assessment of ecotoxicological responses of zooplankton communities to a simulated oil-spill and subsequent remediation practice. Shoreline enclosures were used to simulate an ecological setting exposed to an oil-spill in a boreal lake, with traditional oil collection

methods being applied followed by enhanced monitored natural recovery remediation practice. Additionally, a novel indicator of normalized vitality (NV), sequence counts of RNA metabarcoding normalized by that of DNA metabarcoding, was developed for assessment of ecological responses. DNA and RNA metabarcoding detected similar taxa richness and rank of relative abundances. NV presented relatively greater magnitudes of differential responses of community compositions to treatment compared to DNA or RNA metabarcoding. NV also differed between Rotifer and Arthropoda, possibly due to differential life histories and sizes of organisms. NV could be a useful indicator for characterizing ecological responses to anthropogenic influence; however, the biology of target organisms and subsequent RNA production should be considered when using this approach for ecological assessment.

1.06.P-Tu002 Wastewater Microorganisms Impact the Microbial Diversity of Stream Periphyton and Its Tolerance to Micropollutants

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Effluents from wastewater treatment plants contain complex mixtures of micropollutants and are of major concern regarding their impacts on the quality of receiving streams. Stream periphyton, a complex community composed of bacteria, algae, fungi and protozoa, plays a crucial role in ecosystem functioning. It has been shown that periphyton tolerance towards micropollutants was increased upon *in-situ* exposure to wastewater. The aim of this study was to determine the role of microorganisms from wastewater effluents in the observed increased tolerance. To achieve this goal, we grew periphyton during 4 weeks in flow-through channels that were continuously alimeted with stream water that was mixed with various fractions of filtered or unfiltered treated urban wastewater. Filtration was meant to remove microorganisms while dissolved nutrients and micropollutants remain unaffected. Tolerance of periphyton to a micropollutant mixture extracted from passive samplers that were immersed in the effluent was determined via short-term bioassays. Impact of wastewater on microbial communities of periphyton was also assessed via sequencing of 16S and 18S rRNA genes in periphyton and compared to stream and wastewater communities. Moreover, 51 micropollutants were analysed in water and periphyton samples. Our results show an increased tolerance for periphyton exposed to unfiltered wastewater, but not to the filtered one. Hence, the removal of ~99% microorganisms from the effluent led to the loss of increased tolerance. Effluent filtration also led to differences in the diversity and composition of periphyton communities compared to the control (without wastewater) and to the communities exposed to unfiltered wastewater. Moreover, the relative contribution of wastewater bacterial communities in periphyton was higher than that of stream communities, especially for periphyton exposed to unfiltered wastewater. These results suggest that microbes originating from the wastewater may have contributed to the increased tolerance, either directly via the colonisation of periphyton by micropollutant-tolerant taxa, or indirectly by modifying species interactions within the community. Overall, our study highlights the need to consider the role of wastewater microorganisms, in addition to in-stream exposure of periphyton to micropollutants, in order to better understand potential impacts on the receiving water bodies.

1.06.P-Tu003 Structural Dynamics of Biofilms Exposed to Contaminants and Effects on River Functions

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During the last decades, intensified human activities and emerging contaminants have been disturbing the quality of freshwaters. Such hazardous compounds threat and alter the physical and chemical properties of aquatic ecosystems and consequently the global biodiversity, biological processes and functional services provided by rivers. Assessing quality of freshwaters is thus became of high importance. In that context, bioindication evolves into an indispensable tool for water quality assessment. However, current biological monitoring relies mainly on techniques which need highly-qualified personnel and are time-consuming as well as costly. It includes for example capture-based surveys and observations for vertebrates and invertebrates, or microscopic observation and plate-counting for microorganisms. The use of environmental genomic approaches would be a good solution to develop a rapid, efficient and cost-effective method due to the development of high-throughput sequencing and bioinformatics tools. The aim of our study is to define biofilm-based indicator(s) to assess metal impacts on rivers using environmental genomic tools. River biofilms represent a highly diversified biota (bacteria, algae, fungi and meiofauna) and have the interesting feature of responding quickly and in a sensitive manner to changes in water quality. Contaminants could have repercussions on biological organization of these biofilms. Because they are at the bottom of the trophic food chain, impacting these microecosystems would affect biogeochemical cycles of major elements in the river and river functions. To that end, glass slides were exposed to increasing concentrations of cobalt (3 µg/L, 30 µg/L and 60 µg/L) in outdoor mesocosms for 28 days. Growing biofilms were collected by scraping the slides after 7 d, 14 d, 21 d and 28 d of exposure. Cobalt bioaccumulation in biofilms will be determined and its potential impacts on their biological organization will be assessed through molecular approaches. Biofilms are currently analyzed at the level of functional genes related to C, N and S cycles. Bacteria and algae abundance is also assessed using qPCR. Future analyses will examine microorganism diversity as well as their interactions. The overall results will bring insights on the evaluation of metal bioavailability and toxicity towards aquatic communities, and are expected to go deeper into the genomic-based tools improvement in environmental risk assessment.

1.06.P-Tu004 Use of Metagenomics to Assess the Impact of Organic Fertilizers in the Agricultural Soils Microbiomes

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The use of organic fertilizers has been proposed as alternative source of nitrogen and phosphorus for agriculture, with the added benefit of compensating the deficit in organic matter of most European agricultural land. This practice constitutes a paradigmatic example for circular economy, as the ultimate residues of food waste or food production facilities is utilized o increment food production. However, there is a growing concern that it also may recirculate different biological hazards into the food circuit, with the consequent risks for environmental and human health. We present here an study about the persistence of the microbiomes from different organic fertilizers (sewage sludge, pig slurry, and composted organic fraction of municipal solid waste), and of the antibiotic resistance genes (ARG) associated to them into agricultural soils and products. We observed that only minor fractions of the organic fertilizers' microbiome persisted into the soil only few weeks after the fertilization, but that this fraction is likely responsible for the increase in ARG loads observed in the fertilized soils. We also observed that both effects (microbiome colonization and increase in ARG loads) were transient, being almost obliterated six months after fertilization. Finally, our data show that ARG and some bacterial forms from organic fertilizers may reach the agricultural produce (lettuce and radish, in this particular study) and, therefore, that adequate waste management and good pharmacological and veterinarian practices are needed to significantly reduce the presence of these ARGs and of potentially pathogenic bacteria in agricultural soils and plant products.

1.06.P-Tu005 Transfer of Antibiotic Resistance Genes From a Waste WATER Treated Plant Effluent to the Filter Feeding Zooplankton *Daphnia magna*

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Antibiotic resistant bacteria from waste water treated plant WWTP effluents are pervasive in aquatic environments. A facilitating role for the emergence of waterborne, multi-drug resistant bacterial pathogens has been attributed to biofiltration but had not yet been substantiated. This study investigated the ability of *Daphnia magna* to reduce antibiotic resistant genes (ARGs) in water and incorporate them. *D. magna* individual juveniles of 4 days old were exposed for 10 days to a WWTP effluent with and without 1 mg/L of the antibiotic doxycycline and then transferred to clean water for an additional 4 days. Experiments were conducted with food: *Chlorella vulgaris* at 5 10⁵ cells/ml, which was cultured in semi-axenic conditions. Results showed negligible effects of the antibiotic on *D. magna* reproduction and successful transfer of several ARGs from WWTP effluents to *D. magna* quantify by qPCR. These included quinolone and sulphonamide resistant genes, blaTEM and blaCTXM.

1.06.P-Tu006 Efficient Reduction of Biohazards From Wastewater by Soil Aquifer Treatment Systems

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Under the actual climate change scenario, circular economy represents a suitable option to reevaluate resources such as water with a minimal waste generation. Among all the different initiatives to water regeneration we find wastewater reuse. However, this is severely limited by its potential chemical and biological risks for users, as well as for the environment. Wastewaters contain variable concentrations of many biologically active compounds, including antibiotics (ABs) and represent a substantial biological hazard, as they may contain pathogens, parasites, viruses, antibiotic resistant bacteria (ARB) and antibiotic-resistance genes (ARGs). It is precisely the simultaneous presence of ABs, ARB and ARGs what makes Sewage Treatment Plant (STP) effluents potentially dangerous hotspots for antibiotic-resistance dissemination. Taking this into account, Soil Aquifer Treatment systems (SAT) represent a cost-efficient option to revalue water due to their potential to reduce biohazards from STP effluents. In this study we analysed the reduction of ARGs and pathogenic bacteria in treated effluent from an operational STP (Palamós, N.E. Spain) using two SAT systems upgraded with reactive barriers, one based on vegetable compost/clay and another one based on woodchips/clay, plus a reference SAT system which operated without reactive barrier, composed only by sand. Using a combination of 16s rDNA sequencing and quantitative PCR techniques, we observed a reduction of measured biohazards similar to or better than the ones reported by other tertiary wastewater methods. Taxonomic analysis of the microbiomes sampled before, inside, and exiting the systems showed an ecological succession of bacterial groups, which correlated to the decrease on both pathogen bacteria and ARG loads. Taken together, the data shows the suitability of using SAT systems as tertiary treatments to reduce biohazards from wastewaters in an effective and economically efficient manner.

1.06.P-Tu007 The Gastrointestinal Microbiota of *Gadus morhua* Callarias Originating From a Chemical Munition Dump Site

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After the First and Second World Wars, several hundreds of thousands tons of munitions containing chemical warfare agents (CWAs) were dumped in the seas. Recent studies have confirmed that CWAs have started leaking from the corroded munitions and their concentrations in the bottom waters are expected to peak in the next decades, which could have serious physiological and ecological consequences for aquatic organisms. In recent years, several studies have documented the toxic effects of CWAs on aquatic species. According to these studies, the condition of fish and macrobenthos inhabiting dumping sites is worse compared to those from non-polluted areas. Moreover, the free-living bacterial communities in the dumping sites are significantly less abundant and diverse. One may expect, that the presence of CWAs would also affect the gut-associated bacterial communities in fish, which is of vital importance for their condition. The aim of this study was to test the hypothesis postulating that Baltic cod collected from the CWAs dump site in the Bornholm Deep have dysregulated gastrointestinal microbiota. In order to verify this hypothesis, metagenomic studies, based on the sequence analysis of the 16S rRNA encoding gene of bacteria present in the gastrointestinal tracts of cod collected from the Bornholm Deep (14 ind.) and a control site (maximally distant from all official chemical warfare dump sites, 10 ind.), were conducted. The results of this study suggest that dwelling in the Bornholm Deep area significantly reduces the taxonomic diversity and affects the composition in the microbiota of Baltic cod, which is likely the result of the vulnerability of most bacterial taxa to the CWAs present in the surrounding water.

1.06 Metagenomics tools to unravel environmental adverse effects on communities (Virtual Only)

1.06.V-01 Keystone Fungal Species Associated with Petroleum Hydrocarbons Bioattenuation in Polluted Niger Delta Soils *Chidinma Peace Okafor¹, Chioma Blaise Chikere¹, Onyewuchi Akaranta¹ and Khayaletu Ntushelo², (1) University of Port Harcourt, Nigeria, (2)UNISA, South Africa*

The microbial diversity index could be used as a reflection of the detrimental effect of a pollutant in the environment. Petroleum hydrocarbons are environmental stressors posing threat to impacted soils of the Niger Delta region of Nigeria. Petroleum hydrocarbon pollution usually alters the physicochemical properties of the soil and in most cases makes the soil very acidic affecting soil microbial diversity. Fungi are known to tolerate harsher environmental conditions than bacteria, hence this study sought to detect fungal species actively involved in petroleum hydrocarbon degradation in the region under very acidic conditions. Enrichment method was used in culture-based isolation of the fungal isolates while shotgun metagenomics with >15M read depth was carried out on duplicate soil samples from 2 different locations; 4.816480°: 6.111690° Oporoma and 5°257.8912: 6°355.5608, Igbuduya community with soil pH of 3.2 and 2.3 respectively. The result shows that compared with unpolluted controls very few fungal genera *Scatylidum*, *Stachybotrys*, *Scedosporium*, *Cladophialophora*, *Cladosporium*, *Aspergillus*, *Talaromyces*, and *Penicillium* were identified from the polluted sites with active genes and metabolic pathways for hydrocarbon degradation. *Stachybotrys* was the only fungus seen across all soil samples investigated hence maybe the keystone fungal genus for hydrocarbon impacted sites of the Niger Delta. This organism could also be used for ecotoxicity testing as well as for bioremediation of the petroleum hydrocarbon impacted soils.

1.07 Novel methods and approaches for assessing effluents and ambient water toxicity

1.07.T-01 The Use of Novel Tests With Non-Standard Species and Methods to Resolve Toxicity As Part of TIEs *William L. Goodfellow¹, Konrad Kulacki¹, and Alexandra Steele¹, (1)Exponent, Inc., United States*

Effluent discharges from municipal and industrial wastewater treatment systems have traditionally been monitored for concentrations of individual chemicals. A substantial limitation to only using chemical specific monitoring is that one needs to know the chemicals in the effluent in order to evaluate their concentrations or have a standard list of chemicals that ensures the net for regulatory compliance is sufficiently broad to protect the quality of receiving waters. To overcome this limitation, as well as to account for the many effluents that contain mixtures of chemicals, whole effluent toxicity testing (WET) or whole effluent assessment (WEA) can more effectively determine whether the effluent is toxic to aquatic organisms in its entirety, rather than by virtue of individual components. Traditional WET or WEA methods use standard test species such as fish, invertebrates, and algae as the analytical detectors to assess whether the effluents are unacceptably toxic even when the specific chemical composition is not immediately known. Should effluent samples be either acute or chronically toxic as determined by WET or WEA tests, an additional strategy termed toxicity identification evaluation (TIEs) is often utilized. TIEs use physical and chemical fractionation procedures to further characterize effluent and to isolate and identify the specific toxicant(s). When non-standard species or novel tests are necessary for the assessment of effluents to account for site-specific considerations of the discharge or receiving stream, one is faced with the challenge of how to further identify the toxicant without the aid of the large chemical toxicity database, like those available for standard species or traditional test methodologies. Therefore, in many instances, when WET or WEA investigations characterize the effluent as toxic, it is difficult to move further, i.e., to identify or remove the toxicant. This presentation will explore strategies to further evaluate effluents while employing novel tests with non-standard species and methods, and still allowing the ability to resolve the specific chemical causing or contributing to the toxic effluent.

1.07.T-02 Critical Review of Effect-Based Methods for WATER Quality Assessment in the Context of Refinery Effluents *Carolin Riegraf¹, Cornelia Kienle² and Etienne Vermeirssen³, (1)Swiss Centre for Applied Ecotoxicology, Switzerland, (2)Swiss Centre for Applied Ecotoxicology, Switzerland, (3)Oekotoxzentrum Eawag-EPFL, Switzerland*

Permitted effluent discharges from refineries may, after required treatment, still release traces of organic and inorganic contaminants, which might pose a risk to the receiving marine or freshwater environments. Often, the contaminants comprise a complex mixture, which is challenging to capture by monitoring only a limited number of chemical parameters and selected pollutants, as currently required by discharge regulations and permits in Europe. Effect-based methods (EBMs) for water quality

monitoring might be considered as complementary elements to chemical analysis in the Refinery BAT (best available technique) reference document as well as in the context of the EU Water Framework Directive. Based on previous research published by Concawe and additional relevant literature, we evaluated several EBMs suitable for monitoring and assessment of refinery effluents. Therefore, the suitability of established EBMs with respect to several criteria *inter alia* commercial availability, general validation maturity, previous application to environmental samples, interpretation of assay results and sensitivity were reviewed. We propose that, for EBM-array testing, samples should be collected directly in refinery effluent discharges using either native composite sampling or (large volume) solid-phase extraction depending on requirements of the selected EBMs. Initially, samples may be collected on a quarterly basis. However, the frequency may be adapted on a later stage depending on the established data basis. Taking into account the above established criteria as well as advantages and disadvantages of the available EBMs, we foresee a mutually supporting and complementary EBM array covering a broad range of refinery effluent specific pollutants as well as various endpoints and trophic levels could be suggested. Such an array might be composed of (i) an acute/chronic growth inhibition assay with green algae, (ii) a chronic water flea assay, both performed in glass vessels, (iii) a luminescent bacteria assay, (iv) the umuC genotoxicity assay and (v) an aryl hydrocarbon receptor activation assay performed in 96-well plates. This five-component EBM array would require validation against different refinery effluents in order to confirm its suitability for a holistic monitoring and assessment of a broad range of refinery effluents. Further, the bioassay selection might be improved according to newly generated data and information aiming to optimize a future EBM-array proposal.

1.07.T-03 Assessment of the Ecotoxicity of Pharmaceutical Residues and Their Removal Options

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Pharmaceutical wastewater is composed of a cocktail of substances including active ingredients, adjuvants and their respective metabolites which can cause toxicity to aquatic organisms. Unfortunately, the degree of degradation of pharmaceutical wastewaters is highly variable, as in general they include some recalcitrant chemicals. We conducted a case study in a company that produces several drugs. The influent had a high COD (20,000 mg/L) and high toxicity ($LC_{50} > 1\%$). The aim was to investigate what technologies can be applied to reduce the ecotoxicity to *Daphnia magna* by operating a pilot membrane bioreactor (MBR) composed of an aerobic/anoxic system, finding that it contributed to the reduction of concentrations of micro-pollutants with removal rates of 88-94%, but ecotoxicity of pharmaceutical wastewater still persisted. After coupling it to nanofiltration, organic matter decreased in the effluent, but the toxicity did not disappear. The combination of MBR with reverse osmosis was able to eliminate both organic matter and ecotoxicity, demonstrating that the effluent treated in this configuration was non-toxic to *D. magna* ($LC_{50} > 100\%$). Pharmaceuticals were identified and measured by liquid chromatography/mass spectrometry with QTOF detection to demonstrate which were the potential causative agents. Another case study applied advanced oxidation processes (AOPs) to wastewater from a pharmaceutical industry producing antibiotics. We found that among the different AOPs (Fenton, peroxon, ozone) the most efficient was supersaturated ozone. Even when antibiotics can produce harmful effects, such as resistance, the most toxic compounds were quaternary ammonium disinfectants used as plant sanitizers. Although the toxicity appeared to decrease, its ozonation residues produced long-term toxic effects. Furthermore, even when the post-treatment with bacterial activated carbon could deem the effluent non-toxic, the metagenomic analysis showed that *Pseudomonas sp.* were the selected bacteria, possibly causing resistant genes. These studies provide a better understanding of the risk of ecotoxicity of pharmaceutical waste for the aquatic environment, highlighting the need to optimize treatment technologies.

1.07.T-04 The Impact of Species Sensitivity on the Bioanalytical Assessment of WATER Quality

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Bioanalytical tools, such as *in vitro* reporter gene assays, are commonly applied in water quality assessment and will likely be augmented to European water regulation soon. Problematically, most suggested bioassays are of mammalian origin and may not be representative of aquatic species. We report the parallel utilization of mammalian and fish reporter gene assays of the xenobiotic metabolism and oxidative stress response to perform a bioanalytical evaluation of the presence and removal efficiency of compounds of emerging concern in wastewater. For the fish, we utilized previously developed zebrafish reporter gene assays of the xenobiotic metabolism in ZFL cells and the oxidative stress response in ZF4 cells. In parallel, HepG2 and MCF7 cells-based reporter gene assays were employed as the mammalian counterparts. Influent and effluent water samples from three Swedish WWTPs were bioanalytically evaluated to derive point estimates ($EC_{IR1.5}$; EC_{10}) and bioequivalents (BEQ). In terms of the oxidative stress response, we found no systematic differences between fish and mammalian bioassays. The derived $EC_{IR1.5}$ values were identical for influent samples and within the same range for effluents. The removal efficiency was somewhat lower for MCF7 than ZF4. However, we observed statistically significant differences in activity induced by the oxidative stress reference compound tBHQ, introducing an up to 5-fold difference in bioequivalent recalculations. Induction of the xenobiotic metabolism response was identical between species for the reference compound TCDD. However, we recorded statistically significant differences in activity between species for the environmental samples, with differences being site-specific. Removal efficiencies were relatively low for the AhR endpoint in both species, with 45-88% per site. The variation in induction patterns between species hints towards the involvement of different toxicokinetic and -dynamic mechanisms. An analysis of the chemical data via iceberg modelling and effect-directed approaches will be conducted to potentially identify the chemical compounds and classes causing alternating responses between species. Conclusively, we depict how different species-based *in vitro* assays might provide different results in water quality assessment, which can impact the calculation of point estimates, points of departure, and bioequivalent values.

1.07.T-05 Androgenic ACTivity of WATER and Sediment Up- and Downstream of a Wetland

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Environmental water contains chemicals including herbicides, personal care products, household products, and pharmaceuticals which disrupt the endocrine system through interacting with the hypothalamic-pituitary-gonadal axis. Many of the synthetic chemicals show androgenic or antiandrogenic activity resulting in disruption of reproductive development in males. Traditionally water quality is determined with targeted chemical analyses. However, these analyses overlook the biological effect of the compounds occurring in mixtures and the possible toxicity of untargeted compounds. *In vitro* bioassays can be used as an alternative to animals to test for changes in a particular cell type that can predict adverse outcomes such as endocrine disruption which is the biological effect and focus of this study. The aim of the study was to investigate whether water and sediment from a small rural town in South Africa contained ligands that will activate/inhibit the androgen receptor (AR) using an *in vitro* reporter gene assay. The MDA-kb2 reporter gene cell line was used. An MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) viability assay was performed to prevent false interpretation of the reporter gene assay. Effluent sampled from the water treatment plant (WWTP) activated the AR (relative effects potency (REP₅₀) = 0.17 ng testosterone-equivalents/μL) while water sampled in the wetland caused AR inhibition (REP₈₀ = 114.5 ng flutamide equivalents/μL). No (ant)agonistic responses were observed for the sediment samples at the concentrations tested. The identity of the compounds in the WWTP effluent should be investigated. The concentration ranges of sediment samples should be expanded to ensure that no AR ant(agonists) are present in the environment.

1.07 Novel methods and approaches for assessing effluents and ambient water toxicity (Poster)

1.07.P-We012 Artificial Infiltration As an Effective Pre-Treatment in Drinking Water Production: An Effects-Based Assessment

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With growing concerns over the deteriorating quality of surface water supplies around the world, groundwater is becoming a more important drinking water source. When groundwater supplies become low, aquifers can be artificially recharged with surface waters. However, surface waters containing contaminants can negatively affect the groundwater quality and should therefore be monitored accordingly. Effects-based bioassays have been applied in the assessment of drinking water; yet very few studies have investigated the artificial infiltration treatment of water using such a bioanalytical approach. The aim of the current study was, thus, to monitor the detection of bioactivities across the entire treatment process of a drinking water system fed artificially infiltrated groundwater. In this system, artificial infiltration of the surface river water takes approximately 6 months. A single sampling event was conducted in the fall season and the samples were enriched by solid phase extraction to a final relative enrichment factor of 50 for the bioanalysis. A panel of cell-based reporter gene assays representing several toxicity pathways was selected: oxidative stress response (Nrf2 activity), aryl hydrocarbon receptor (AhR) activation, and hormone receptor-mediated effects (e.g., estrogen receptor [ER], androgen receptor [AR]). AhR and ER bioactivities were detected in samples collected from the river intake and in the open-air storage basins prior to artificial infiltration. However, the AhR activity decreased and ER activity was effectively removed following artificial infiltration. While the specific chemicals inducing the AhR activity in this study are not known, the AhR is considered an important receptor in multiple physiological functions. In the Nrf2 and AR assays, no bioactivities above cut-off levels were detected in any samples collected along the entire treatment process of the drinking water production from source to tap. Using a suite of bioassays, the current study highlighted the effectiveness of artificial infiltration in reducing bioactive compounds in this raw river water. Further, we demonstrated that *in vitro* bioassays are useful for screening a broad range of effects in drinking water.

1.07.P-We013 Assessment of Online-Biomonitoring Systems to Monitor Varying Wastewater Effluent Compositions

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Wastewater treatment plants (WWTPs) are a major source of micropollutants to surface waters. To generate knowledge about the presence, potential effects and removal of micropollutants it is a valuable approach to monitor treated wastewater effluent of WWTPs. Currently, monitoring is based on grab or composite samples of the effluent. These samples cannot capture the transient nature of micropollutants in the wastewater. Especially in WWTPs with industrial input, the wastewater composition can be highly variable and a continuous testing would be advantageous, but very labour and cost intensive. A promising concept is to monitor the wastewater effluent by automated online-biomonitoring systems to detect harmful substance peaks. By applying living organisms to constantly monitor water quality, the systems are designed to raise an alarm if the organism's responses exceed a harmful threshold, which can trigger further chemical monitoring or mitigation measures. Currently online-biomonitoring systems are established for drinking water and surface water but only few experiences exist for wastewater. This study demonstrates that an online-biomonitoring battery using algae (*Chlorella vulgaris* in the Algae Toximeter, BBE Moldaenke), water flea (*Daphnia magna* in the DaphTox II, BBE Moldaenke) and amphipods (*Gammarus pulex* in the Sensaguard, WALTER Tecyard) can be adapted for wastewater surveillance. We showed that a back-washable membrane filtration system proved to be indispensable for adequate preparation of treated wastewater for continuous low-maintenance

operation of the online-biomonitoring systems. Only minor deviations in the reaction of the organisms towards treated and filtered municipal wastewater compared to culture medium could be detected. When treated wastewater was spiked with peak concentrations of selected model compounds, such as diuron, chlorpyrifos and sertraline, the organisms exposed in the online-biomonitoring systems showed distinct responses. The observed variety of responses between the systems showed the value of using a battery of online-biomonitoring systems to detect substances of different mode of actions. Strong behavioural changes after spikes did trigger an alarm, whereas only minor ones did not. However, even in those cases, the observed single reaction patterns of the test organisms provided relevant information about the influence of the test substances within the wastewater. To further support the understanding of the reactions of the test organisms exposed to changing wastewater composition, the effects on feeding activity of *G. pulex* were evaluated in parallel and results indicated significant differences between the treatments. Overall, these findings provide an important basis showing that online-biomonitoring systems have the potential to act as alarm systems in the wastewater sector.

1.07.P-We014 Evaluation and Standardization of a Short Term Chronic Method Using *Daphnia magna*

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In 2009, US EPA's Office of Research and Development (ORD) published a paper wherein a 4-d static-renewal survival and growth test was developed for use with *Daphnia magna*. The test results were compared to performance criteria and results from 7-d survival and reproduction tests with *Ceriodaphnia dubia*, to compare the level of sensitivity between the two methods. Results from tests using the 4-d *D. magna* survival and growth method indicated that this method produced consistent results with CVs < 20% with various reference toxicant materials and provided data that were both reproducible and useful for detecting potential toxicity in aquatic environments. Since that publication, there has been an increased interest in using the *D. magna* method in Whole Effluent Toxicity (WET) tests associated with NPDES permits where source water chemistry, i.e. conductivity, may be more appropriate for *D. magna*. In 2020, ORD began re-evaluating the method in a bi-laboratory approach at US EPA laboratories in Cincinnati, OH and Duluth, MN as well as a collaboration with National Council for Air and Stream Improvement (NCASI). During the early testing phase, it was decided to look at influence of different foods and amounts on growth, in addition to revising the test performance criteria from the final control weight being 10 times higher than the initial weight, to one that is based on a minimum final weight criterion. Various food combinations: alfalfa and algae; yeast, cerophyl, trout chow (YCT) plus algae; and flaked food, alfalfa, yeast (FFAY) plus algae were tested in different concentrations to see which gave a consistent final weight. Once the final food and amount was chosen, tests were conducted with three reference toxicants, NH₄Cl, ZnSO₄ and diazinon, using newly released young < 8 hr old with six replicates per treatment. This presentation will highlight the latest progress of the different food and rationing as well as reference toxicant testing.

1.07.P-We015 Microbial Fuel Cells and Constructed Wetlands As a Sustainable Alternative for the Treatment of Hospital Laundry Wastewaters: Assessment of Load Parameters and Genotoxicity

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The present research investigated the sequential use of microbial fuel cells (MBFC) and constructed wetlands (CW) for the treatment of wastewaters generated at a hospital located in a city of the Rio Pardo Valley, Brazil. The MBFC was vegetated with *Chrysopogon zizanioides* whereas *Hymenachne grumosa* was planted in the CW unit. The hydraulic retention time in each unit was of 7 days, totaling 14 days of treatment. The evaluation of the efficiency of the integrated system considered the analysis of load parameters and the genotoxicity potential by the comet assay with *Daphnia magna* before and after the treatments. Regarding the raw wastewaters, the obtained results revealed that the mean values of some of the analyzed parameters were above the maximum emission limits established by Brazilian and international guidelines. Furthermore, the untreated effluents presented a strong genotoxic effect ($p < 0.001$) against *D. magna*. On the other hand, the results of the treated wastewaters showed reductions of 79.8%, 78.6%, 81.6%, 53.1%, 81.5% and 99.9% for COD, BOD₅, Total N, TDC, DOC and Turbidity. Also, the treated wastewaters presented significant reductions ($p < 0.05$) of the genotoxicity. Therefore, the use of the integrated system showed that both units had complimentary effect and presented satisfactory results regarding the removal of the organic load, complying with national and international standards and significantly reduced the genotoxicity. Furthermore, besides being effective, the integrated system can be sustainable from both environmental and economic aspects, especially for developing countries, where there is limited or insufficient wastewater treatment infrastructure.

1.07.P-We016 Novel Application of Machine Learning and Image Analysis to Automate the Sea Urchin Embryo Test

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Since more and more xenobiotic compounds are being found in the environment, ecotoxicology faces an overwhelming challenge in the identification of toxicants. The combination of high-throughput *in vivo/in vitro* bioassays with high resolution chemical analysis is an effective way to elucidate cause-effect relationships. However, these combined strategies imply an enormous workload that can hinder their implementation in a routine analysis. The purpose of this study was to develop a new high-throughput screening methodology based on image analysis and machine learning to ease the application of the sea urchin embryo bioassay in complex toxicant identification pipelines such as effect-directed analysis. A training set of 242 images was used to calibrate the larvae size-increase and malformation level classification. Two classification models based on partial least squares

discriminant analysis (PLS-DA) were built and compared. Moreover, Hierarchical PLS-DA shows a high proficiency in classifying the larvae, achieving a prediction accuracy of 84% in validation. The scripts built along the work were compiled in a user-friendly standalone app (SETApp) freely accessible at <https://github.com/UPV-EHU-IBeA/SETApp>. The SETApp was tested in a real case scenario to fulfil the tedious requirements of a WWTP effect-direct analysis.

1.07.P-We017 Miniaturized Acute *Daphnia magna* Assay for the Testing of Low-Volume Samples in the Scope of Monitoring Environmental Contamination

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The regular monitoring of surface- or groundwater quality is most often done on the basis of chemical analysis and ecological assessments. But chemical analysis or chemical monitoring is not a 100 % save assessment method for the overall toxicity of environmental samples as usually not all substances are analyzed and each and every non-analysed substance may exert toxic effects. Ecotoxicological analysis may hence be a supplemental method because the overall toxicity of all bioavailable substances are included. But as most ecotoxicological tests with organisms were originally developed for the assessment of waste water or single chemicals (e.g. EU-REACH regulation) the volumes and replicates used and needed are normally quite large as restriction of volume or numbers of replicate tests was and is not an obstacle. In case of a regular monitoring of water samples the sample volumes for single tests are often restricted because a battery of several tests is used, and at times water samples are concentrated (enriched) which further reduces the available sample volume. In contrast to chemical analysis biological or ecotoxicological tests often need volumes above 200 mL per sample. This may be one reason why ecotoxicological tests such as the acute *Daphnia* assay is not as often used as it might be beneficial. To overcome this obstacle here a miniaturised daphnid assay protocol usable for day-to day monitoring is proposed. As a basis, we used the publication by Grintzalis et al., (2017) for a follow-up literature review. From this literature review three approaches to achieve a reduction in sample volume seemed feasible: reduce the ratio volume-per-neonate, reduce the number of concentrations tested or reduce amount of neonates per replicate or concentration. Subsequently, we adapted the standard acute daphnia test based on the OECD standard to our specific low volume monitoring requirements using water extracts. Multi-well plates were used which had the additional advantage of easier evaluation of daphnia mobility via microscopic inspection. Finally, we confirmed the applicability and sensitivity of our new system by comparing the toxicity values from the standard assay and the miniaturized assay for different compounds either retrieved from literature data or from lab tests. Our approach is likewise applicable for other samples with restricted availability, such as microplastics, and contributes to a reduction in chemical waste for laboratory tests.

1.07.P-We018 A Method for Testing Impact of Pesticides on Amphibians Behavior Larvae

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According to the International Union for Conservation of Nature (IUCN), amphibians are suffering a massive decline. With more than 40% of endangered species, they represent the most threatened group among vertebrates. Amphibians decline is multicausal and pollution is usually considered as one of major factors involved in population decrease and local extinction. Among parameters tested in amphibians' ecotoxicology, molecular biomarkers and morphological traits are commonly measured and compared between tested concentrations. A less investigated branch of amphibians ecotoxicology is the impact of toxic molecules on amphibians behavior. Indeed, although toxicants impacting the nervous and/or the endocrine systems are likely to indirectly impact behaviors such as activity, hardiness or exploration capacity which are known to influence survival and reproduction in amphibians, only few research focused this adverse effect and no standardized protocol were developed for demonstrating and quantifying this assumptions. In this work, we developed an 8 days protocol aiming at evaluating impacts of pesticides on *X. laevis* larvae swimming behavior. Briefly, we exposed freshly fertilized eggs to different concentrations of Chlorpyrifos, an organophosphate pesticide. Embryos were individually exposed from NF stage 8 to 192 hours post-fertilization. From day 6 to day 8, animals swimming behavior was recorded once a day for 10 minutes and the trajectory was extracted using the open-source R package, Bemovi. From this trajectory were extracted the number of recorded moves, the step speed and the total distance traveled during the test. Our results showed the efficiency of our method to demonstrate impacts on larvae swimming behavior components. In addition, this work highlighted a time-dependent impact of exposure on activity parameters suggesting the need of longer protocols and represents an opening toward studies on impact of pollutants to amphibians personality.

1.07.P-We019 The Amphibian Short Term Assay (ASTA)

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According to the International Union for Conservation of Nature (IUCN), amphibians are suffering a massive decline. With more than 40% of endangered species, they represent the most threatened group among vertebrates. Amphibians decline is multicausal and pollution is usually considered as one of major factors involved in population decrease and local extinction. Especially, the use of pesticides in the context of farming activities is suspected to degrade the quality of breeding sites (e.g. lac, swamp, and ponds). Indeed, these molecules are carried by surface and sub-surface runoff as well as drainage into aquatic systems and can be found in high concentrations in surface waters. Freshwater ecosystems are of crucial importance for amphibians' life cycle. In pond-breeding amphibians, embryonic and larval development takes place in aquatic habitats. Environmental conditions experienced over aquatic development are critical since they affect growth (rate and timing) and survival until metamorphosis.

They also have far-reaching consequences for postmetamorphic performances as they influence size and body condition at metamorphosis, two factors directly related to fitness components (survival, growth, and reproduction) at terrestrial stage. Therefore, exposure to chemicals during aquatic development may have a strong detrimental impact on amphibians' population dynamics. In this work, we developed a protocol covering not only the embryonic phase but also 4 days of the larval phase. This method aimed to evaluate impacts of pesticides on molecular traits in addition to morphological and life history traits. Briefly, we exposed *X. laevis* freshly fertilized eggs to different concentrations of Chlorpyrifos, an organophosphate pesticide. Animals were individually exposed from NF stage 8 to 192 hours post-fertilization (hpf) during which three common molecular biomarkers and morphological traits were individually measured at different times. Our results demonstrated the efficiency of our method to evaluate physiological, morphological and life-history traits changes with significant statistical decrease in Acetylcholinesterase activity and body length at 192hpf in addition to growth rate. Besides, in accordance with literature, our results demonstrate a higher response of 192hpf larvae compared to 96hpf embryos, thus re-evaluating the relevance of tests based on embryonic phase only.

1.07.P-We020 Evaluation of the Ecotoxicological Impact of Cigarette Butt Littering in Marine and Freshwater Environments

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Cigarette butts (CBs) represent the most littered item in the world and one of the most diffused personal waste on beaches and coastal areas in general. CBs can entrap a wide range of dangerous chemical compounds, such as PAHs, hydrocarbons, trace elements and nicotine, produced both by tobacco combustion and treatment during cultivation and manufacture. The aim of the present work was to evaluate the effect of CB leachates on target species with two batteries of marine and freshwater ecotoxicological bioassays. Marine tests showed an inhibition of growth with the two different marine algal species (*Phaeodactylum tricornerutum* and *Dunaliella tertiolecta*), an inhibition of bioluminescence in the bacterium *Aliivibrio fischeri*, an alteration on the correct early development of the serpulid *Ficopomatus enigmaticus* larvae and immobilization of *Acartia tonsa* individuals at both 24 and 48 hours of exposure. Freshwater tests showed again an inhibition of the bioluminescence with *Aliivibrio fischeri* together with a significant stimulation of radical apparatus elongation in two different superior plants (*Cucumis sativus* and *Lepidium sativum*), while *Sorghum saccharatum* showed no statistically significant differences with controls, even if a biostimulation was again registered. Similar results were obtained with the freshwater microalga *Raphidocelis subcapitata*, which showed biostimulation of algal growth. Two other assays were performed with freshwater crustaceans, *Thamnocephalus platyurus* and *Daphnia magna*, underlining no significant differences with controls with both species. With *D. magna*, also the heartbeat rate on adults exposed to different concentrations of freshwater samples was calculated. A significant increase of the bpm in adults exposed to high concentrations of smoked CB leachate was observed after 48 h of exposure. In conclusion it was possible to define a preliminary scenario of the different effects that CB litter can cause to different key organisms in aquatic environments, underlining the importance of monitoring of this "emerging pollutant" in both internal and coastal areas.

1.07 Novel methods and approaches for assessing effluents and ambient water toxicity (Virtual Only)

1.07.V-01 A Novel Floatation-Based Sample Processing Method for Raw Wastewater Testing

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Wastewater testing presents an opportunity to track infectious agent spread in human populations if an appropriate assay can be developed to assess large fluidic volume samples including sludge component. Currently, challenges associated with filtration-based workflows include throughput and requirement of pre-processing steps. The COVID-19 pandemic has highlighted the need for simple but sensitive technologies and workflows to enable environmental monitoring for communicable disease agents. Human biofluids such as urine, stool, or blood are found in sewage and present an efficient pooled-sample source to monitor infectious agents in at the community level. Here we present a workflow enabling assessment of large volumes of wastewater without the need for pre-processing such as the removal of solids from sludge. The approach uses buoyant, functionalized microbubbles for nucleic acid binding. This extraction step is followed by sample clean-up using RNA-binding columns and qRT-PCR. This workflow achieves greater sensitivity analyzing >75% smaller wastewater aliquots, relative to commonly practiced centrifugal ultrafiltration-based methods (i.e., 3-10 mL vs. 45 mL wastewater). Microbubbles are buoyant, hollow glass microspheres (< 20 µm in size) which can be functionalized to capture analytes. Microbubble enabled workflows are not limited by sample volume or container shape and have minimal equipment requirements. Using microbubbles for nucleic acid extraction employs a positive selection protocol in which the microbubbles bind to the desired target – in this case, total RNA including SARS-CoV-2 if present – isolating and enriching the analyte for downstream analysis (e.g., qRT-PCR, next-generation sequencing). This work shows that microbubble nucleic acid extraction can directly interrogate large fluidic volumes of unfiltered raw sewage samples to increase RNA capture for downstream processing.

1.07.V-02 AMPHITOX: A Customized Early LIFE Stage Toxicity Test for a Holistic Assessment

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The aquatic environment is recipient, transporting medium and sink for the majority of xenobiotic chemicals. In order to compare results from different matrices we develop in the early 90s, AMPHITOX as a customized early life stage, sensitive, cost-effective, biological test methods. By reporting acute, short term chronic and chronic results in Toxicity Units it was possible to quantify with the same parameter the toxicity of water, sediments, industrial effluents, soils and leaches establishing thresholds of toxicity

as they can affect biodiversity richness, e.g. the presence in the water column and sediment of normal, sensitive, tolerant and very tolerant species directly related to the amount of toxicity. By reporting teratogenesis both for environmental samples and physico-chemical agents, it was possible to provide information of toxic effects on cell differentiation and morphogenesis as well as disruptions like hydropsy and reduction on oxygen consumption. COVID-19 pandemic provided the possibility to explore potential synergistic effects of SARS-CoV-2 and some high production volume chemicals like Ni, Cd, atrazine, 2,4-D and Bisphenol A. For instance, Bisphenol A, 2,4-D and atrazine could produce osmoregulatory disruption resulting in hydropsy that could be associated to ACE2 receptor expressed in early embryonic stages. ACE2 was associated to intra-alveolar edema and is the main multiorgan entrance point of SARS-CoV-2. Hydropsy was reported in COVID-19 fetus. The same chemicals plus Ni and Cd could be related to COVID-19 severity by means of their effect on oxygen consumption. It is well documented that hypoxia triggers the hypoxia inducible factor (HIF) and hypoxia associated with COVID-19 could imply an over-activation of the HIF system influencing the functionality of 200 genes, including those that regulate apoptosis. Morphological shapes of apoptotic like cells can be observed as toxic effects of different chemicals. Customized early life stage toxicity test provide a huge amount of information from lethality to dysfunctions that contribute to understand the complex effects of physical and chemical agents on living organisms, which seems to include a potential synergistic effect with infectious agents. Considering the increasing complexity of environmental pollution they can contribute as sentinel organisms to a holistic risk assessment towards environmental and human health protection.

1.07.V-03 Developmental Effects of MDMA in Zebrafish - Preliminary Data

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Illicit drugs are a global problem with significant direct and indirect adverse impacts on human health and on wildlife. Among them, MDMA (3,4-methylenedioxymethamphetamine), commonly known as ecstasy, is a synthetic drug with psychotropic effects and in some countries, it is used in psychotherapy for post-traumatic stress disorder. Human urinary excretion of MDMA and its metabolites contribute to their presence in waste water treatment plants (WWTP) and in water bodies. Because WWTP are insufficient to remove these compounds thus low and high concentrations have been reported in water bodies and ID are suspected to be harmful to exposed aquatic organisms, including fish. Early development stages are prone to adverse effects caused by contaminants or drug consumption in humans, presenting seasonal characteristics related with social behaviour and tourism. Zebrafish (*Danio rerio*) have semi-transparent embryo and consequently this species has been used as a key model to study developmental biology [2] and namely to assess toxic effects during early stages of *embryonic development* [3]. The aim of this work was to evaluate the MDMA effects on embryonic development of *D. rerio*. For that, embryos with 2-3 hours post-fertilization (hpf) were randomly exposed for 96 h to different concentrations of MDMA (0.02, 0.2, 2, 20 and 200 µg/L) to evaluate mortality, larvae size, eye area, yolk area, oedema area, head area, tail curvature and number of malformations. Preliminary data showed that MDMA exposure have no significant effects during embryonic development, for all the endpoints assessed and for all the 5 concentrations tested. The MDMA embryo exposure, in this study, showed no teratogenic effects. Nonetheless, our result agrees in part with Barenys et al. (2021) study, which observed no general developmental delay or general toxicity [1] but their observed the increased in spontaneous tail coiling in zebrafish embryos at 24 hpf and a faster hatching rate [1]. Our data indicated that MDMA is not teratogenic and do not affect mortality, larvae size, eye area, yolk area, oedema area, head area, tail curvature and number of malformations but more studies are important to assess MDMA effects in zebrafish embryo. Funding: This work was supported by National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020 and PTDC/CTA-AMB/6686/2020.

1.07.V-04 Evaluation of the Ecotoxicity of Two Solvents to *Pelophylax perezi* and *Xenopus laevis* Tadpoles

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Organic solvents are commonly used in ecotoxicity assays as carriers to promote the dissolution of hydrophobic compounds (e.g., some pesticides, pharmaceuticals, polycyclic aromatic hydrocarbons). In this context, the selection of the solvent and the concentration to be used in bioassays is of most importance because itself may induce toxicity to the biota. Existing guidelines, namely those published by the Organisation for Economic Co-operation and Development (e.g. OECD Guidance document 23, 2019), recommend that the maximum concentration of solvent to be used should not go over 100 µL/L (0.01%), so that the effects observed during the assay are solely a consequence of the exposure to the chemical under study. Acetone and acetonitrile are two solvents widely used in ecotoxicity assays as they can dissolve several chemicals, though only a few studies assessed their lethal and sublethal effects to freshwater biota, namely to aquatic life stages of amphibians. Therefore, this work aimed at evaluating the lethal and sublethal toxicity of these two solvents to tadpoles of two anuran species: the standard species *Xenopus laevis* (autochthonous from sub-tropical/tropical regions) and *Pelophylax perezi*, a species autochthonous from temperate regions (Iberian Peninsula and South France). For this, tadpoles at developmental stages NF46 (for *X. laevis*) and G25 (for *P. perezi*) were exposed, for a period of 96 hours, to 9 and 7 concentrations, for acetone (0.125 to 5%) and acetonitrile (0.0625 to 5%), respectively. Mortality of tadpoles was monitored at each 24 h exposure period and at the end of the assay the following endpoints were also assessed: total length; snout-to-vent length; inter-orbital distance and weight difference. The obtained results revealed that acetone caused mortality above 20% at concentrations equal or above 2 and 5% while acetonitrile at concentrations equal or above 0.25 and 1%, respectively for *X. laevis* and *P. perezi*. As for sublethal effects, acetone induced significant alterations at 1 and 1.5% and acetonitrile at concentrations above 0.25 and 0.0625%, for *X. laevis* and *P. perezi*. These results suggest that

higher concentrations of the two studied solvents may be used in short-term toxicity assays when studying compounds with very low solubility in water.

1.07.V-05 Risk Assessment of Selected Veterinary Pharmaceuticals in Aqueous Solutions and Livestock Farms' Whole Effluents

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Pharmaceuticals are extensively used in livestock agricultural production for enhanced productivity and profitability. A consequence of this is the release of residues of original parent compounds and their metabolites into the environment. Literature suggests that residues of some pharmaceuticals induce toxic effect on non-target organisms. Ecological and human health risk assessments of selected pharmaceutical compounds in livestock farms effluent and surface water samples were conducted using different bioassays including algal growth inhibition, acute/chronic toxicity, Ames mutagenicity and oestrogenic activities tests. Results showed that pharmaceutical cocktails exerted significant observable effects on the growth (inhibition) and yield of *Pseudokirchneriella subcapitata* relative to controls. Algal response to exposure to cattle and poultry effluents indicated compromised water quality characteristics. Neonates of *Daphna magna* exposed to environmental concentrations of pharmaceutical compounds had 100% mortality in undiluted and 1:2 dilutions after 48 h. All samples tested were "highly acutely toxic"- the toxicity units (TU) values for at least one of the tests fell between 10 and 100 TU. Mutagenicity was 6 to 8-times the natural background rate; low carcinogenic risk was observed for 17 β -estradiol (2.4 μ g/L) especially, via possible accidental ingestion during recreational activities. Cattle and poultry farms effluents were mutagenic, piggery, sheep farm effluents were oestrogenic, and the piggery effluent had the highest toxicity.

1.07.V-06 Temporal Variation of EcoToxicological Risks in Agricultural Ditches

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Traditional chemical monitoring of water bodies fails to accurately assess and explain ecotoxicological risks, because it does not incorporate the combined action of the complex chemical mixtures present in the aquatic environment. Therefore, there has been an increasing interest in the combination of passive sampling and effect-based methods (bioassays) to identify ecotoxicological risks. Yet, only few studies have used effect-based methods to address temporal variations in toxic pressure. The aim of the present study was, therefore, to assess the temporal variation of ecotoxicological risks in agricultural ditches, and to identify the drivers of these risks by combining passive sampling, bioassays, and chemical analysis. Four locations in the province of Friesland, The Netherlands, were sampled during four consecutive continuous sampling periods. Silicone rubbers sheets and polar organic chemical integrative samplers (POCIS) were exposed in the water bodies for the passive sampling of hydrophobic and hydrophilic organic compounds, respectively. Subsequently, six *in vivo* and sixteen *in vitro* CALUX[®] and antibiotic-activity bioassays were exposed to extracts of the passive samplers. For ecotoxicological risk interpretation, the bioanalytical responses were compared to effect-based trigger values (EBTs). Temporal variation in the identity and concentrations of chemicals was observed at all locations. Six bioassays showed responses above their EBTs and EBT exceedances were observed at all locations and during all sampling periods. Evaluating the joint responses of the entire bioassay battery, distinct differences in bioanalytical response profiles between locations and sampling periods became clear. Temporal variation in ecotoxicological risks was well captured by combining passive sampling and bioanalytical tools, but was poorly explained by target chemical analysis, confirming that ecotoxicological risks were largely caused by the combined action of unmeasured chemicals. To become adequate and future-proof, regular chemical water quality assessment strategies should thus incorporate passive sampling and effect-based methods. Dedicated suspect- and/or non-target analysis may subsequently identify the chemical drivers of the ecotoxicological risks. It is concluded that timing of effect-based monitoring in future monitoring programs should be carefully considered, since this has considerable influence on the chemical water quality assessment.

1.07.V-07 The Impact of COVID-19 Lockdown on the WATER Quality of River Matanza-Riachuelo, Buenos Aires, Argentina

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Matanza Riachuelo River, originates some sixty kilometers west of Buenos and from the city's earliest days, the river has been a dumping ground for waste and sewage becoming one of the most polluted waterways in the world. Close to five million people live in the Riachuelo's basin. The toxicity of environmental samples from potentially polluted and reference stations were evaluated from the 90's by means of the AMPHITOX test including surface and ground water, leaches, industrial effluents and soils. The data, expressed in acute, short-term chronic and chronic Toxicity Units (TUa, TUsc and TUC) resulted in a maximal value of 1000 TUC, found in a leach, while the lower toxicity value was 1.4 TUa corresponding to two surface water samples. In reference places no toxicity was found. On 3 March 2020, SARS-CoV-2 was confirmed to have spread to Argentina and a nationwide lockdown was established in all the country from 10 May until 17 July, where the lockdown was due to be gradually loosened to lead to the return to normality. In 17 May 2020 surface water samples were obtained from 4 locations along the Matanza-Riachuelo River. The first in the intersection of Route 4 with River Matanza (conductivity 956 μ S/cm²; oxygen 6,4mg/L; pH 5,90), the second in the intersection with General Paz (conductivity 1230 μ S/cm²; oxygen 5,4mg/L; pH 6,24), the third in Victorino de la Plaza Bridge (conductivity 1730 μ S/cm²; oxygen 5,7mg/L; pH 6,20) and the last one in la Boca close to Nicolas

Avellaneda Bridge (conductivity 830 μ S/cm²; oxygen 5.9mg/L; pH 6.43) close to the Riachuelo discharge in Rio de la Plata. Those data imply some level of activities in spite of the lockdown. The water samples were evaluated for toxicity with *Danio rerio* (zebrafish) embryos. 10 embryos by duplicate in 10 mL were maintained in zebratox solution with a constant temperature of 28 C and a maximal exposure time of 240hr. No significative lethality was registered compared with control groups. The results point out that within a few days after the lockdown the water quality improved significantly to the point that no lethal effects were registered even for chronic exposure. It also implies that the highly polluted sediments did not polluted the water column to a toxicity level. Thus, by preventing the discharge of toxic materials in toxic amounts, the river could recover basic condition to sustain a wide range of biodiversity contributing to the further recovery of the watershed towards sustainability

1.08 Understanding and linking effects of contaminants in wildlife across multiple biological levels

1.08.T-02 Revealing the Modes of Action of Hg(II)-Induced Modulatory Effects on the Immune Responses in Waterfowl Upon Viral-Like Immune Challenge With In Vivo Experiments

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Trace metals such as mercury (Hg) have been reported to be immunotoxic. Our previous field study showed that even low environmentally relevant levels of Hg exposure affected the immune responses in Arctic Barnacle goslings (*Branta leucopsis*) upon a viral-like immune challenge, mainly via inducing inflammation and impairing B-cell functioning. To verify the results from the field study and give more insights on the causality of Hg-induced immunomodulatory effects, we conducted a controlled *in vivo* experiment with Pekin ducklings (*Anas platyrhynchos domesticus*) as a model waterfowl. Day-old ducklings were exposed to five different levels of Hg(II) premixed in the feed up to 1.00 mg Hg(II)/kg feed for 23/24 days. There were 12 ducklings in each exposure group. 24h before termination, 50% of the ducklings in each exposure group were challenged with 50 μ g/kg body weight (b.w.) poly I:C via intraperitoneal (i.p.) injection, while the other 50% of ducklings received saline solution (0.9% NaCl). On day 23/24, ducklings were euthanized and samples including liver, spleen and plasma were collected. Our results showed that the internal total Hg (tHg) concentrations in the liver of ducklings were strongly correlated with the concentrations in the feed, and nicely covered the range of Hg concentrations found in gosling livers from the field study. The major effects of Hg(II) exposure in ducklings were modulated gene expressions in the spleen, i.e. a downregulation of miR-155 suggesting impaired B-cell functions, and an upregulated expression of iNOS, which could be an indicator for inflammatory effects. Therefore, the findings from the *in vivo* duckling experiment were in line with the field study, and these adverse effects may lead to an affected immune competence at the individual level and a higher risk of infections.

1.08.T-03 Evaluating the Impact of Pesticide Dermal Exposure on Terrestrial Amphibians

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Amphibians are globally considered the most threatened group of vertebrates, with anthropogenic pollution being regarded as one of the main factors contributing to their decline. In their terrestrial stages, they possess a very distinctive skin that is highly permeable to chemical substances and that is involved in a plethora of physiological processes. Therefore, and because these animals frequently occur in agricultural areas, the impact of pesticide application on non-target amphibians is highly relevant. In this study, we investigated the toxicity of dermal exposure to two current-use pesticides, the pyrethroid insecticide alpha-cypermethrin, both as an active ingredient and as a formulation (Fasthrin 10 EC), and the herbicide metsulfuron-methyl, on amphibian terrestrial stages, using Perez's frog (*Pelophylax perezi*) as a model species. To do so, embryos of *P. perezi* were collected and grown in the laboratory until their terrestrial phases. Juvenile frogs were exposed by overspray using a pump calibrated to simulate realistic pesticide application regimes. During the experimental period (7 or 21 days) we recorded survivorship and changes in body condition, as well as analysed histopathological changes on dorsal skin. Besides, we collected samples from their skin secretions to investigate skin peptide bioactivity, and frog tissue samples to examine pesticide residue toxicity accumulation by LC-MS. Results reveal that overspray with alpha-cypermethrin-based insecticides at realistic doses compromises survival of juvenile frogs, and that acute toxicity of the formulation is increased relative to that of the active ingredient. Mortality among individuals exposed to metsulfuron-methyl was observed in the second half of the monitoring period, although no significant differences with the control group were detected. Regarding effects on the skin, we observed changes in the skin epithelium of juvenile frogs exposed to either alpha-cypermethrin or metsulfuron-methyl. The most prominent alteration consisted of an increased stratification and swelling of keratinocytes in the *stratum corneum* (the outermost layer of the amphibian skin). A thicker epidermis could impact the permeability of the skin and affect skin functionality, possibly compromising animals' fitness. Considering the importance of terrestrial amphibian stages in maintaining population dynamics, further attention should be paid to assessing pesticide risks for these stages.

1.08.T-04 Negative Relationship Between Glyphosate Exposure and Female Reproductive Parameters in Iberian Hares (*Lepus granatensis*)

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Important changes occurred during the 20th century in farmland ecosystems due to an important homogenization of the landscape caused by the reduction of field margins and to the use of a large amount of pesticides and fertilizers. This change in agricultural management has been linked to the loss of plant and wildlife biodiversity. In the specific case of pesticides, there are significant gaps in the knowledge of their secondary or sub-lethal effects on wildlife. Under this context, the aim of this study was to evaluate the relationships between pesticide exposure and reproductive parameters in females of Iberian hare (*Lepus granatensis*), as a sentinel species of the potential effects that the use of pesticides can have on the wildlife of agrarian ecosystems. To carry out this study, residues of 8 pesticides in the gastric content of hunted female hares along with different reproductive parameters were measured in two hunting areas free of pesticides (control 1 and 2, n=18) and from two areas where pesticides are used (treated 1 and treated 2, n=32). Hares from the two pesticide-treated areas showed high prevalence of glyphosate (22.2 and 31.6%; treated 1 and 2, respectively) and in one of these areas (treated 2) also of 2,4-D (12.5%). Fungicides were detected in some individuals (6.6%), and insecticides were not detected. No residues were detected in hares from pesticide-free areas. Hunted females from pesticides-treated areas showed a significantly lower ovarian size and weight than hares from pesticides-free areas. Significant differences were also detected among study areas in the number of atretic follicles, being higher in individuals from one of the pesticide-treated areas (treated 2). Overall, results showed a higher prevalence of pesticide and lower reproductive condition in females from pesticide-treated areas, which in the long-term could have effects at the population level.

1.08.T-05 mRNA Expression-Augmented Contaminant Biomonitoring in a Seabird Using a qPCR Array

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Birds can bioaccumulate persistent contaminants, and maternal transfer to eggs may expose sensitive embryos to high concentrations, but using tissue residue concentrations alone to infer whether adverse effects are occurring suffers from uncertainty and is a major limitation in many biomonitoring programs. Furthermore, efficient and sensitive biomarkers to assess effects caused by contaminants remain limited or unvalidated in many avian wildlife sentinel species, although mRNA expression-based methods are promising. Therefore, we are evaluating spatial and correlational relationships between embryonic contaminant concentrations (total mercury, 20 organochlorine pesticides, 17 perfluoroalkyl substances, 35 polychlorinated biphenyls, and 22 halogenated flame retardants) and the mRNA expression of 24 genes associated with hepatotoxicity in embryonic liver tissue from a wild-collected Pacific Ocean Alcidae seabird, the rhinoceros auklet (*Cerorhinca monocerata*). Fresh, unincubated eggs were collected and artificially incubated in the lab. Pre-hatch embryos were sampled for hepatic tissue, from which total mRNA was extracted, reverse transcribed to cDNA, and assayed by qPCR (²??Cq). The remaining embryo carcass was analyzed for total mercury and organic contaminants by mercury analyzer and GC-MS, respectively. Contaminant concentration data and qPCR relative normalized gene expression data is being analyzed with linear models to conduct spatial comparisons of contaminant concentrations and relative normalized gene expression, as well as evaluating correlative relationships between contaminants of concern and relative normalized gene expression. This study provides valuable baseline data on contaminant concentrations in wild seabirds in northwestern North America, and examines the utility of gene expression approaches for use in contaminant biomonitoring and assessing the effects of potential environmental disasters (e.g., oil spills).

1.08 Understanding and linking effects of contaminants in wildlife across multiple biological levels (Poster)

1.08.P-Tu008 VKORC1L1 Gene Expression in Barn Owl (*Tyto alba*) and Common Kestrel (*Falco tinnunculus*) From Different Environmental Settings

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Anticoagulant rodenticides (ARs) have been widely used to control rodent populations. The intensive use of ARs causes frequent secondary exposure of non-target wildlife species. The exposure of birds of prey to ARs is of particular interest as they are top predators and may be used as sentinel species. Previous studies have shown that these species may be highly sensitive to ARs. These compounds inhibit the enzyme vitamin K epoxide reductase complex (VKORC) leading to the animal death due to internal haemorrhages. In birds, sensitivity to ARs and also the main component of this VKORC activity vary among species. For example, the main contributor for VKORC activity in chicken is the VKORC1L1 while in turkey it is the VKORC1 but little is known about its regulation in birds of prey. In the present study, we hypothesize that the VKORC1L1 gene expression is differentially expressed in individuals breeding in different environmental settings related with the use of ARs. To test this hypothesis we sampled individuals of a nocturnal species, the barn owl (*Tyto alba*), and a diurnal species, the common kestrel (*Falco tinnunculus*), which potentially feed on rodents in different areas -varying in relation to land uses- in the Region of Murcia (Spain). For gene expression analysis we used blood samples of adults and nestlings. The results showed a differential tendency in the expression of the VKORC1L1 gene respect to land use in both age classes. Interestingly, a 3-fold increase was found in owlets of barn owl breeding in an intensive farming landscape. This tendency was similar in common kestrel chicks. Though preliminary,

the results of this study will therefore allow to better understand the mechanisms of sensitivity to ARs and the intra- and inter-specific differences in the response to these toxic compounds. *Acknowledgement* - This research was funded by a charge of the Ministry for Ecological Transition to INIA (EG17-017), the project PID2019-108053RJ I00/AEI/10.13039/501100011033 funded by AEI, and Seneca Foundation (20945/PI/18). LS supported by 21413/FPI/20. Fundación Séneca. Región de Murcia (Spain) and EIDUM. SE supported by IJCI-2017-34653.

1.08.P-Tu009 PFAS Toxicity in Birds: Transcriptomic Response and Association With Fatty Acid Composition in Experimentally Exposed Canaries

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The widespread use of Per- and poly fluoroalkyl substances (PFAS), together with their persistence and bioaccumulation potential, resulted in global contamination of the environment, wildlife and humans. Despite the ubiquity of PFAS, the toxicological and biological effects of these compounds are not well characterized yet. Several studies have been conducted near the 3M fluorochemical plant in Antwerp using songbirds. These studies revealed the highest PFOS levels ever found in wildlife. Furthermore, levels of other PFAS (PFDS, PFHxS and PFOA) were also the highest ever reported in bird eggs. We assessed the effects caused by PFAS in a model species (canaries (*Serinus canaria*)), at environmentally realistic concentrations, across different levels of biological organization. For this purpose, canaries were exposed during a month to water treated with PFOS and PFOA (the two PFAS most frequently detected). Two exposed groups and a control group were included in the experiment. Each experimental group consisted of 30 birds (15 males and 15 females) We studied the transcriptional response of canaries using high throughput sequencing of adipose tissue. This enabled us to assess the molecular key events that initiate the toxicity pathway of these compounds. In addition, using small amounts of blood, we characterized exposure and effect biomarkers (i.e. immune system assays and fatty acid composition), enabling us to establish linkages between key events in the biological cascade. Transcriptomic analyses revealed dysregulation of 1249 transcripts (711 overrepresented and 538 underrepresented relative to controls), showing a clear dose-response to PFAS exposure. Using the annotation from chicken genome, we identified several metabolic pathways significantly affected by PFAS treatment, including lipid, glycerolipid, and sphingolipid metabolism, the Krebs cycle, and the apoptosis pathways. These results can be interpreted as a reduction in lipid catabolism and the activation of programmed cell death, suggesting degeneration of the fatty tissue due to a lipid metabolism disruption by PFAS. In relation to this, we found that exposed females, but not males, presented a lower percentage of unsaturated fatty acids in their red blood cells. All tests were performed using non-destructive samples, which is crucial to reproduce the study in wild birds. This is the first use of whole-genome transcriptomics to analyze the toxic effects of PFAS in songbirds.

1.08.P-Tu010 Exploring the Pollutant Exposure and Its Potential Health Implications in Common Eiders Through Nontarget and Target Analyses

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The common eider (*Somateria mollissima*) is a large sea duck found in arctic and temperate regions of the northern hemisphere. Only the females incubate the eggs and they fast for the 26-day long incubation. The resultant loss of body mass has the potential of remobilizing pollutants stored in body tissues. The population of common eider is declining dramatically in Finland with a concurrent shift in the adult sex ratio towards a heavy male bias. While predation of breeding females factors into this, the role of pollutant exposure deserves further investigation as the Baltic Sea is among the most heavily polluted seas in the world. To this end, we collected paired blood samples from females ($n = 28$) in early and late incubation as well as complete clutches from ten nests in spring 2021 at a colony in southwestern Finland. We have already analyzed the blood samples using untargeted metabolomics and nontarget screening of pollutants at Aarhus University. The same blood samples as well as egg samples will be used for targeted analyses in January-February 2022 at the Norwegian University of Science and Technology of organophosphate flame retardants, UV filters, bisphenols, and per- and polyfluoroalkyl substances (PFASs). These results will be presented at the conference.

1.08.P-Tu011 MicroRNAs: A Link Between Pollution and Disease in Migratory Shorebirds?

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Immunomodulation has been linked to chemical pollutant exposure in various species. Yet, the molecular mechanisms underlying the effects of chemicals on the immune system are not well understood. Recently, microRNAs have been identified as post-transcriptional regulators of gene expression and key modulators of the immune system. MicroRNAs are short noncoding RNAs that bind semi-complementary to messenger RNA to prevent their translation into proteins. Changes in microRNA expression have been found after exposure to various chemicals and pathogens (e.g., influenza virus). The presence and stability of microRNAs in biological fluids, including serum, makes them promising, minimally-invasive biomarkers of immunomodulation. In this study, we performed microRNA sequencing of serum samples from shorebirds that migrate along the East-Asian Australasian Flyway (EAAF). Many EAAF shorebird populations are declining, but the role of pollutant exposure in these declines is unclear. The aim of this study is to elucidate the role of microRNAs in pollutant-induced immunomodulation by

comparing serum microRNA profiles with (i) avian influenza (AIV) infection status and (ii) blood pollutant levels. Serum samples from avian influenza-infected (n=8) and non-infected (n=6) ruddy turnstones (*Arenaria interpres*), captured in Australia in 2019, were analysed for microRNAs using next-generation sequencing. Reads were aligned against the ruddy turnstone genome and known microRNA sequences from MiRBase were quantified using miRDeep2. Differential expression analysis was carried out using EdgeR and potential microRNA targets were identified using RNAhybrid. We identified 117 known microRNAs of which four were differentially expressed (DE) in AIV-infected ruddy turnstones. All four DE microRNAs are regulators of immune pathways. Furthermore, eight of the identified microRNAs, including the DE miR-204/211, target AIV genome sequences. These findings might reflect an antiviral response, supporting the notion that serum microRNAs have potential as biomarkers for immunomodulation in avian wildlife. Blood pollutant levels (per- and polyfluoroalkyl substances and heavy metals) and oxidative stress biomarkers are currently being quantified and their influence on microRNA expression will be assessed, to further elucidate the role of microRNAs in pollutant-induced immunomodulation. All results will be presented at the conference.

1.08.P-Tu012 Pollution in the Coastal Wetlands of East Asia and the Decline in Migrating Shorebirds - First Results of the Coast Impact Project

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The main purpose of the project is to investigate the influence of pollution and infectious diseases on shorebirds that migrate along the East Asian–Australasian Flyway (EAAF). Many shorebird populations are in severe decline along the EAAF, the reasons for which are largely unknown. Climate change and habitat destruction are considered important contributors to these declines, the role of pollution thus far remaining understudied. Pollutants might nevertheless have an important role to play in shorebirds being in dire straits, potentially affecting the food on which they rely, and having direct and indirect (e.g. through immunomodulatory properties) effects on their survival. COAST IMPACT investigates the impact of pollution on the availability and biodiversity of benthic macroinvertebrates (food resources to shorebird) in coastal wetlands in East Asia, and the resulting combined impact of food availability, pollution, and disease on migrating shorebirds using these wetlands as stop-over sites. In 2018-2021, about ten locations along the Chinese coast were visited to study the abundance and biodiversity of benthic macroinvertebrates and to obtain sediment samples and macroinvertebrates for contaminant analysis. Contaminant analysis for per- and polyfluoroalkyl substances (PFASs), persistent organic pollutants and other emerging contaminants were conducted. In addition, PFASs analysis, and Total Oxidisable Precursor Assay to determine PFASs not amenable to standard analysis were also performed on a limited number of samples. PFASs were analysed in blood samples from several shorebird species with differently reported population statuses (some in severe decline while others are more stable) in Taiwan and Australia. For Australian birds also their avian influenza infection status was assessed. POPs were generally low in all sediment and macrobenthos samples with the vast majority of samples below the quantification limit. The Bohai Sea was shown to potentially be a hotspot for contamination with PFASs and other emerging contaminants (e.g bisphenols and parabens). In addition, the macrobenthos is declining in most of the studied sites. Regarding shorebirds, birds from Taiwan showed significantly higher concentrations than birds from Australia. The reported population status seems to be correlated to total PFASs concentrations in birds sampled in Taiwan. Also, birds with high concentrations of PFASs had a higher chance of being infected with avian influenza.

1.08.P-Tu013 Pesticide Toxicity and Farmland Bird Populations in England

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Agricultural intensification has increased efficiencies and altered farming practises and, the use of pesticides and fertilisers have contributed to this *green revolution*. However, there have been widespread effects of this transformation. In face of these changes, several non-target farmland species have declined. Extensive research has presented the dangers these agrochemicals pose to invertebrates such as pollinators, but less is known about organisms at higher trophic levels. Previous modelling studies have linked the decline of farmland birds with the use of pesticides. Although, many of these are limited as only a single pesticide class or species is considered in the study. This study considers the wider range of pesticides utilised on arable farmland with the aim to better understand the driver behind the immense decline in farmland bird abundance in England. Generalised linear mixed models were used to test for spatio-temporal associations between agricultural usage of 16 pesticides and changes in populations of 30 species (farmland and generalist) between 2007 and 2016. The abundance of half of the farmland species included in the study were negatively associated with one or more pesticide and this was also similar amongst the generalist species. Farmland species have been at risk since the drastic agricultural changes have been introduced but generalist species in farmland areas are also under threat. Although it isn't possible to infer that pesticide application is the causal factor of these declines observed in the findings, the study has highlighted three pesticides that potentially pose the greatest risk and may warrant further research (cyprodinil, glyphosate and prothioconazole). We can conclude that pesticide application has a varying effect across the different species included in this study, but it is also likely that there are multiple factors contributing to the effects we have observed that were not considered here and should be the focus of future research.

1.08.P-Tu014 Does Domestic Pesticide Use Influence Bird Abundance in Private Gardens?

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Urban greenspaces bring a range of advantages for residents, being associated with improved mental health and well-being, which was even more essential during the lockdowns of 2020 and 2021. Domestic gardens are also a crucial component of cities. In the UK, approximately 87% of households have gardens; this represents about 23 million gardens covering 400,000 ha. Domestic gardens are highly heterogeneous but collectively support considerable biodiversity and ecosystem services such as pollination. Nonetheless, garden pesticide utilisation is often let aside when assessing the risk to wildlife from chemicals. We investigated if the use of domestic pesticides and/or veterinary medicine on pets (cats and dogs) has an impact on the abundance of bird species in private gardens. However, collecting data in private gardens can be arduous in normal times and doubly during a global pandemic. This is why we cooperated with the Garden Bird Watch (GBW) scheme of the British Trust for Ornithology (BTO) by emailing a questionnaire to volunteers on their pesticides usage, cat and dog use of flea and tick treatment. We also obtained the garden information and data on abundance of bird species for the years 2020 and 2021. We obtained data for 733 participants, of which 32% used garden pesticides, and 78.6% and 76.8% treated their dogs and cats, respectively, for fleas and ticks over the last year (at the time of survey). The herbicide glyphosate was the most used garden pesticide (20% of households surveyed). Preliminary analysis found negative relationships between the use of pesticides in gardens with the abundance of certain common garden bird species, such as blackbird (*Turdus merula*), house sparrow (*Passer domesticus*), starling (*Sturnus vulgaris*), or greenfinch (*Carduelis chloris*). Additionally, we explored the impact of urbanisation on bird abundance in private gardens and found that a significantly higher number of birds were recorded in rural areas than in urban areas, but this was probably explained by the general size of gardens, with rural gardens being bigger than urban gardens.

1.08.P-Tu015 Assessing the Utility of Nuptial Pad Morphology for Predicting Reproductive Success in Wild Anuran Populations (*Rana temporaria*)

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Amphibians are undergoing declines on a global scale. Pollutant induced reproductive toxicity ('reprotoxicity') is thought to be a major contributor to amphibian declines, however, evidence linking reprotoxic effects to population declines is lacking for amphibians. One obstacle for assessing reprotoxicity in wild amphibian populations is the lack of informative biomarkers for pollution exposure with relevance to reproductive health. One candidate biomarker for males is a secondary sexual characteristic, the nuptial pad. Here, we investigated the utility of nuptial pad morphology for predicting mating success in wild male common frogs (*Rana temporaria*) in the UK. Sampling sites were located in Devon (2015, 2020) and in Scotland (2021). On arrival at each pond site, once active breeding had been confirmed by observing pairs of frogs in amplexus, 1-6 replicate tubs (66 cm diameter circular, black plastic) were set-up. Pond water (25L) was added to the tubs, along with 8 males and 2 females. This generated a 4:1 male:female sex ratio, which mimics natural conditions in common frog populations. Breeding behaviour was observed every 45 minutes for each replicate tub and at each successive observation, the identity of the male frog(s) in amplexus was recorded. Breeding data collected from a total of 8 different pond sites in Devon (27 tubs in 2015, 3 tubs in 2020) and 4 pond sites in Scotland (8 tubs in 2021). We found that in all years, male frogs that won the competition for the female more commonly had a longer nuptial pad, with an overall level of 61 % across all years ($n = 76, p < 0.001$). Most winning male frogs had a darker nuptial pad for two of the three study years, with an overall level of 63 % across all years ($n = 76, p < 0.001$). These data suggest that features of nuptial pad morphology are a predictive indicator of mating success in male common frogs. Given that nuptial pads have been identified in all anuran amphibian species analysed so far, nuptial pad morphology has potential for use more widely as a biomarker of male reproductive capability across a broad range of amphibian species.

1.08.P-Tu016 Investigating the Resilience of Native and Invasive Amphibian Species to Salinisation by Mapping Their Coastal Distributions Across the UK

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The salinisation of freshwater ecosystems is a widespread threat to global biodiversity and ecosystem health. Salinisation occurs when excessive salt, most commonly sodium chloride (NaCl), enters a freshwater ecosystem. The two main causes of salinisation of freshwater are road de-icers and seawater inundation, itself caused by climate change induced sea level rise and/or increased frequency of extreme weather events such as storms. Freshwater vertebrates, including amphibians, typically experience negative impacts of salt pollution, with effects ranging from mortality to developmental delays and abnormalities. Apart from a couple of known salt tolerant species, such as the crab eating frog (*Fejervarya cancrivora*), laboratory exposures have demonstrated that many amphibian species are negatively impacted by salt pollution. However, there are now an increasing number of reports of amphibians inhabiting brackish water suggesting that salt resilience in amphibians may be more common than initially thought. Using citizen science sampling followed by environmental DNA (eDNA) species identification, here we have identified the occurrence of the six native amphibian species (common toad, *Bufo bufo*; natterjack toad, *Epidalea calamita*; common frog, *Rana temporaria*; great crested newt, *Triturus cristatus*; smooth newt, *Lissotriton vulgaris*; palmate newt, *Lissotriton helvetica*) and the invasive alpine newt (*Ichthyosaura alpestris*) in freshwater or brackish ponds in coastal areas across the UK. Volunteers provided three water samples, spaced three weeks apart, starting when they first observed spawn/adult amphibians in their respective water bodies. In addition to the eDNA analyses, the salinity of the water samples was also recorded, in order to align species

distributions with actual salinity levels. The species distribution data will allow us to investigate the extent of amphibian occupancy of coastal water bodies in the UK as well as the tolerance of individual species to salinity in the wild.

1.08.P-Tu017 Investigating the Relationship Between Bioaccumulated POPs and Biomarker Response in Spinner Dolphins, *Stenella longirostris*

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In the Brazilian coast, the spinner dolphin, *S. longirostris*, occurs predominantly at oceanic waters, concentrating at the upper continental slope, with occasional sightings at higher depths or along the continental shelf. Due to the overlap of its distribution with oil and gas extraction and production (E&P), *S. longirostris* was included as a priority sentinel species to be used in the ongoing Cetacean Monitoring Project (PMC-BS) carried out by Petrobras at the Santos Basin pre-salt province (25005'S 42035'W, 25055'S 43034'W). This study investigated the relationship between blubber burdens of polycyclic aromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs) and biomarker response measured in the tegument of *S. longirostris*, sampled within the Santos Basin pre-salt province. Skin and blubber samples (n=16) were obtained opportunistically through remote biopsy, during telemetry or visual survey efforts. Blubber samples were analyzed for PAHs and POPs (PCBs, PBDEs and organochlorines). Skin samples were used to quantify glutathione S-transferase (GST) activity, cytochrome P450 1A (CYP1A) protein content and transcript levels of aryl hydrocarbon receptor (AhR), cytochrome P450 1B (CYP1B), estrogen receptor beta (ESR2), heat shock protein (HSP70) and UDP-glucuronosyltransferase (UGT1). To investigate the relationship between bioaccumulated PAHs and POPs and biomarker response, generalized linear models (GLMs) were constructed. Best fitted models indicated that PAHs levels contributed significantly to the observed variance of biomarker data. In particular, PAHs levels were negatively associated to CYP1B, ESR2, HSP70 and UGT1 transcript levels. Apart from PAHs, only mirex was negatively associated with AhR transcript levels and PBDEs were positively associated with UGT1 transcript levels. Regarding to PAHs, the predominance of negative associations may be due to the prevalence of two and three ring compounds, which have been shown to inhibit biomarker response, such as EROD and GST activity in other species. The negative relationship between PAHs and ESR2 also suggests a potential inhibitory effect over reproductive signaling. Taken together, our results suggest that the levels of bioaccumulated contaminants found in these samples, especially PAHs, may be of concern to *S. longirostris* local population and reinforce the need to continually monitor this species in the Brazilian coast.

1.08.P-Tu018 Polycyclic Aromatic Hydrocarbons (PAHs) in Cetacean From Western Mediterranean Coast

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Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants derived from the combustion of organic matter, highly lipophilic and present in all the world's seas and oceans, as well as in the atmosphere and soils. However, the analysis and impact of these compounds on cetaceans are scarce. Cetaceans, which are considered as oceanic sentinels of human and wildlife health, have been suggested to be especially susceptible to the toxic effects of PAHs. The aim of this work was to detect and quantify 16 PAHs in blubber samples from 58 individuals of various endemic cetacean species stranded on the Murcia coastline (Western Mediterranean) between 2011 and 2018. Only six of the 16 studied compounds were detected with detection frequencies ranging from 17.24% (anthracene) to 98.28% (phenanthrene). The concentrations detected are in line with those obtained by other authors in marine mammals from areas with high anthropogenic pressure. Phenanthrene was the PAH showing the highest concentrations (maximum 205.14 µg/kg lw.), followed by naphthalene, acenaphthene, fluoranthene, fluorine and anthracene. Our results in striped dolphin (the species with the largest number of samples, n=40) followed the same distribution pattern than others striped dolphins elsewhere, dominated by naphthalene (46.86 ± 27.48 µg/kg). This compound is used as a precursor in many industrial processes, as well as a component of pesticides and fuels, so high levels in the study area, characterized by its intensive agriculture (up to 10.9% of the total pesticides used in all of Spain), were expected. On the other hand, the profile of PAHs detected in the study area is mainly composed of low molecular weight compounds, which have the lowest carcinogenic and mutagenic potential. However, some studies have demonstrated the greater genetic susceptibility of Mediterranean cetaceans to the adverse effects of PAHs. Therefore, toxic effects affecting the status of studied populations should be addressed. This is the first work assessing PAHs concentrations in cetaceans from the Region of Murcia, so the data presented in this work could serve as a reference for future research.

1.08.P-Tu019 Organochlorine Pesticides, Polychlorinated Byphenils (PCBs) and Polybrominated Dyphenil Ethers (PBDEs) in Seven Mediterranean Endemic Cetacean Species

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Mediterranean Sea is considered as a hotspot for several persistent organic pollutants (POPs), whose potential health end-points include the reproductive, immune and endocrine systems, and are able to cause cancer. Status of most populations of Mediterranean cetacean species is not generally considered to be favorable and chemical pollution is commonly suggested as a probable cause. Long-term biomonitoring of these cetacean populations provide useful information on the POPs-pollution status of western Mediterranean Sea, which might have direct impact in wildlife and human health. We used blubber from 7 different species of cetaceans (n=57) stranded along the Murcia coastline (SE Spain) between 2011 and 2018 to study 16 different

organochlorine pesticides (OCPs), 18 polychlorinated biphenyl (PCB) congeners and 8 polybrominated biphenyl ethers (PBDE) congeners. DDE and methoxychlor accounted for more than 80% of the OCPs in all species. Mean DDE/tDDT ratio was over 0.9 for all the studied species, which indicates the absence of recent exposures to DDT in these individuals. PCB pattern was dominated by PCBs 180, 153 and 138 and was homogeneous between species. On the other hand, PBDE pattern was dominated by BDE 47, although the rest of the composition was highly variable among species. Striped dolphin was the species showing the highest concentrations of most OCPs (tDDT 4752 ± 7415 mg·kg⁻¹ lw.; methoxychlor 2263 ± 2920 mg·kg⁻¹ lw.), PCBs (6490 ± 9550 mg·kg⁻¹ lw) and PBDEs (73 ± 103 mg·kg⁻¹ lw), generally followed by bottlenose dolphins and common dolphin, which could be explained by their feeding behavior in shallower waters closer to the coast. No significant differences were found among sexes and length was generally not significantly correlated with any compound, although higher concentrations were obvious for older individuals. We lacked enough samples to assess temporal trends. The magnitude of the pollutant concentrations reported in this work was generally lower than those reported for cetacean species in the western Mediterranean. This fact could be explained either by a temporal decrease of these pollutants or different analytical procedures. Although all of these compounds have been banned from decades ago, they are still found at detectable concentrations in cetacean tissues. However, the concentrations found in this study are below those toxicity thresholds reported for marine mammals by different works.

1.08.P-Tu020 Release of Nickel Particles From Mining ACTivity in New Caledonia: Organs Disruption on Eels, *Anguilla marmorata*

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Anthropic activities such as open pit mining amplifies the natural erosion of soils leading to atmospheric emission of metal particles including nickel (Ni). New Caledonia is particularly affected by Ni mining activities because of the presence of ultramafic soils (30% of its surface), which are highly concentrated in Ni. These particles produced during extraction, by atmospheric transport and soil erosion will end up by deposition or leaching in aquatic ecosystems. These deposits can directly impact living organisms in the rivers downstream the mines, such as eels. Despite alarming freshwater Ni concentrations, no study explained so far the consequences of metals contamination on eels living under mining influence, fish known to be sensitive to this kind of pollution. The aim of this study was thus to determine by different approaches how eels, *Anguilla marmorata*, are impacted by Ni contamination and other associated metals by measuring: (i) morphometric parameters; (ii) expression level of genes encoding proteins implicated in lipid metabolism, oxidative stress, detoxification and apoptosis in liver, kidneys, brain, gills, spleen and muscle and (iii) metal concentrations in liver, kidneys, gills and muscle. The results showed that for eels living in rivers downstream mines, liver seems to be the main affected organ with an oxidative stress, lipid metabolism disruption, mitochondrial dysfunction and carcinogenic markers activated. The organ in which Ni was the most accumulated was the kidney. These results underlined the potential toxic impact of metal contamination from mining activity on eels. This study should allow us to define in an integrated way (i) to what extent the contributions of metals related to the mining activities impact the aquatic organisms and (ii) what would be the levels in natural environment tolerable to preserve the wild fauna.

1.08.P-Tu022 Searching for Novel Biomarkers of Effect of Neuroactive Compounds: A Study on Caged Carp (*Cyprinus carpio*) Exposed to Municipal Wastewaters In Situ

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Contamination of the aquatic ecosystems by neuroactive compounds (NCs), such as neuroactive pharmaceuticals, illicit drugs, stimulants and pesticides with neuroactive action, lately emerged as an important environmental issue. However, assessment and identification of ecological impacts of NCs in aquatic ecosystems still faces challenges and limitations [1]. One of them is the lack of sensitive and reliable biomarkers of effect of NCs, which could be considered as valuable early warning signals of environmental contamination by NCs. In our study, we searched for novel biomarkers of effect of NCs by measurement of expression of selected genes (RQ-PCR analysis), encoding proteins involved in neurotransmitter pathways and exocytosis, myelination, neuroendocrine regulation of reproduction and changes in membrane potential, in brain tissue of caged common carp (*Cyprinus carpio*) exposed *in situ* to untreated municipal wastewater and industrial effluents. Among the tested genes, inhibition of serotonin receptor 1aa (*htr1aa*) and myelin basic protein (*mbp*), and stimulation of tachykinine 3a (*tac3a*) and voltage-gated Ca⁺⁺ P/Q channel $\alpha 1a$ (*cacna1a*) was detected. These elements can be considered as promising novel biomarkers of effect of NCs, whose sensitivity and specificity should be also tested *in vitro* and *in vivo*. To link the observed responses to possible adverse outcomes, they were integrated in the Adverse Outcome Pathways (AOPs) available in AOPwiki database. The corresponding Key Event (KE) was identified only for the inhibition of the serotonin receptor gene expression (serotonin receptor inactivation; AOPs 221, 222, 224, 225) implying to depression and agitation as resulting adverse outcomes. We also stress the importance of synchronisation of biomarker research with further development of the AOP framework database, which should include additional events related to disturbance of neural function, as crucial for development of improved biomarker-based strategy for impact assessment of NCs in the aquatic environment. [1] Kaisarevic S, Vulin I, Tenji D, Tomic T, Teodorovic I (2021) *Environ Sci Eur* 33:115 **Acknowledgements:** The research was supported by the EU FP 7 project SOLUTIONS (Grant No. 603437) and The Science Fund of the Republic of Serbia, PROMIS (Grant No. 6061817), BIANCO. The abstract content is the responsibility of the Faculty of Sciences University of Novi Sad, and it does not reflect the opinion of the Science Fund of the Republic of Serbia.

1.08.P-Tu023 The Ongoing of a Histology Atlas of Normal Medaka Growth As a Future Baseline in Histopathology: Part II

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Medaka (*Oryzias latipes*), a small freshwater teleost, is a versatile vertebrate model in aquatic (eco-)toxicity studies. Numerous reports that describe the internal medaka anatomy have focused on the histopathological analysis of target organs at specific developmental periods disregarding a holistic dynamic vision of their normal configuration. However, a thoughtful search surprisingly indicated limited histological information of normal medaka development. Here we describe a histological study of the orange-red strain of medaka with a general exhaustive description of its normal internal anatomy that focuses on two developmental periods: i.e. immature 10 days post-hatch (dph) and maturing 60 dph, selected from the seven developmental periods considered (i.e. 2, 5, 10, 15, 30, 60, and 110 dph) to complete its development. The two developmental periods included in this presentation are an addition to the 2 and 110 dph periods that we presented at the 19th PRIMO Meeting in Matsuyama, Japan. Specimens were collected at the selected stages, overdosed with anesthetic, whole-fixed in Bouin alcoholic solution, embedded in paraffin blocks to obtain parasagittal, coronal and cross sections (7 µm-thick), and then stained with hematoxylin and eosin. This contribution, as part of the aforementioned series of developmental periods, is aimed to provide a comprehensive reference guide for the three-dimensional organization of internal medaka anatomy along its development offering a baseline from which to compare deviations at specific stages of the medaka life. This work was made possible by Spanish Government Grant RTI2018-096046-B-C21 funded by the MCIN/AEI/ 10.13039/501100011033 and the ERDF.

1.08.P-Tu024 Applying MOLECULAR Tools to Assess Metabolic Characteristics in Wild Marine Tursiops truncatus From Different Subspecies

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The knowledge of ecological characteristics of cetacean species is crucial to the design of targeted and effective conservation efforts. In Southern Brazil, two subspecies of bottlenose dolphins (*Tursiops truncatus truncatus* and *Tursiops truncatus gephyreus*) present a parapatric distribution, with the first presenting offshore (OF) and the second coastal (CO) habitats. While stable isotopes values suggest a distinct habitat use for the two groups, no studies have evaluated physiological or biochemical characteristics of the two groups. Therefore, this study aimed to investigate if OF and CO *T. truncatus* present distinct molecular profiles regarding lipid metabolism, immune system and xenobiotic detoxification, further elucidating the extent of habitat overlap between the subspecies and their exposure to anthropogenic pollutants. To fill the gap, skin samples were obtained through remote biopsy from *T. truncatus* in the coastal and offshore areas of the Santos Basin. The latter samples were obtained during the sampling efforts of the “Santos Basin Cetacean Monitoring Project”, carried out by PETROBRAS to satisfy environmental constraint of the federal environmental licensing of PETROBRAS’ activities of production and outflow of oil and natural gas in Santos Basin, conducted by IBAMA. The tegument samples were used to evaluate the transcript levels of fatty acid synthase complex 1 (*FASN 1*), ceramide synthase 3 (*CERS3*), and fatty acid elongases (*ELOVL3* and *4*), markers of lipid metabolism, interleukin 1 alpha (*IL-1a*), and major histocompatibility complex II (*MHCII*) as immune markers, as well as *MT2A*, a metal chelator. Among the target genes, only *MHCII* transcript levels did not vary between the subspecies. The transcript levels of *MT2A* were significantly higher in CO dolphins, suggesting higher metal exposure in the coastal areas. Higher *IL1a* transcripts of CO dolphins indicates higher immune activity, probably associated to their usage of areas impacted by domestic sewage, a source of pathogenic microorganisms. Regarding genes involved in lipid metabolism, *FASN1* transcript levels were higher in OF dolphins, while *CERS3B* and *ELOVL4* transcript levels were higher in CO animals. Such results suggest differences in the lipid composition of skin/blubber from CO and OF dolphins with a more prominent synthesis of ceramides and very long chain fatty acids in CO dolphins, which might alter the susceptibility for increased bioaccumulation of lipophilic pollutants.

1.08.P-Tu025 Different Responses to Chemical Contamination of Three Flatfish Species in the Bay of Seine (France)

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Flatfishes live in contact with the sediment which accumulates many contaminants. Moreover, they represent an important fishery resource. Their exposure to contaminants and their economic significance justify their use as sentinel organisms in marine ecotoxicology. Flounder and dab are two of the most studied flatfish species in relation to chemical contamination in the North-East Atlantic. Sole is one of the main flatfish species distributed all along the French mainland coast. France is currently implementing a program (SELL) to monitor the effects of chemical contamination on marine organisms for the evaluation of criterion 2 of descriptor 8 in the European Marine Strategy Framework Directive. As part of this program, flatfishes belonging to three different species (sole, dab and flounder) were trawled in September 2018 in the Bay of Seine. The objective was to compare biomarker levels and chemical impregnation in these three species present in the same area. Concentrations of metal trace elements and organic contaminants were measured in fish as well as biomarkers reflecting various biological effects of the contaminants. The comparison of these parameters among species revealed more differences than similarities. Sole often differed from the two other species, e.g. regarding silver concentration in liver, the capacity of metabolism of some organic contaminants and DNA strand breaks (comet assay). These results have to be interpreted in relation to the biological and

ecological characteristics of the species and will serve to make better use of sole in the environmental assessment of marine ecosystems.

1.08.P-Tu026 In Vitro Neurotoxic Effects of Emerging Flame Retardants at Non-Cytotoxic Concentrations on Neuroblastoma Cells

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Since 2004, and due to their concern on human health, mainly for neurotoxic effects, some legacy flame retardants (FRs) were restricted or removed from the European markets. Both organophosphorus FRs (OPFRs) and novel brominated FRs (NBFRs) replaced them because they are presumably safer and less persistent emerging FRs. However, as they are included in a commercialized-mixture products, little is known about the potential toxicity and mechanisms of action of individual FRs. Chronic exposure to OPFRs (such as Tris(1, 3-dichloro-2-propyl) phosphate (TDCIPP) and triphenyl phosphate (TPhP)), as well as NBFRs (such as Bis(2-ethylhexyl)tetrabromophthalate (BEH-TEBP) and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB)) is currently occurring in indoor environments. The present study was aimed at assessing the acute neurotoxicity potential of TDCIPP, TPhP, BEH-TEBP and EH-TBB. Human neuroblastoma SH-SY5Y cells were exposed to non-cytotoxic levels of these 4 FRs from 2.5-20 µM during 2-24h. Reactive oxygen species (ROS) response, immune function and neurodevelopment were studied by the expression of nuclear factor erythroid 2-related factor 2 (NRF2), Matrix Metalloproteinase 9 (MMP-9) and Brain Derived Neurotrophic Factor (BDNF), respectively. Furthermore, Sirtuin 3 (SIRT3) and Cytochrome c oxidase subunit 4 (COX-4) expressions were evaluated to study the mitochondrial damage was assessed. Both the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Adenosine triphosphate (ATP) release assays confirmed the cell viability after exposure to the 4 FRs at selected doses and time-points. The results showed that NBFRs (BEH-TBP and EH-TBB) impair both immune response and neurodevelopment as well as induced mitochondrial damage at 24 hours for selected doses, but not OPFRs (TDCIPP and TPhP) which exhibit slightly modulations. The results of the present study suggests the neurotoxic potential of these 2 NBFRs. In further studies, we will integrate obtained results to *in silico* models that extrapolate data into *in vivo* results to better understanding the neurotoxicity of these FRs.

1.08.P-Tu027 Analysis of MOLECULAR Biomarkers in the Digestive Glands of *Unio Crassus* As a Useful Tool for Assessing Pollution of Aquatic Ecosystems: A Study in the Mrežnica River (Croatia)

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The Croatian karst river Mrežnica has been in some parts exposed to pollution from various types of anthropogenic activities, especially from industry. Although most industrial plants now provide proper treatment and discharge of wastewater, pollutants previously accumulated in sediments may still pose a threat to aquatic organisms. Molecular biomarkers are considered the most sensitive and earliest response to pollutants. Therefore, it is useful for pollution assessment to obtain information about the harmful effects of pollutants on biota by analysing different biomarkers. As benthic inhabitants and filter-feeding animals, mussels are capable of accumulating large amounts of pollutants and are therefore considered good bioindicator organisms for assessing water pollution. In order to assess water pollution and evaluate possible effects of pollutants on aquatic biota, a set of molecular biomarkers was analysed in the digestive glands of *Unio crassus* mussels sampled in spring 2021 from two sites at the Mrežnica River: the industrial zone of the Town of Karlovac (contaminated site) and a location upstream from the Town of Duga Resa (reference site). The set included following biomarkers: metallothioneins (MT) for metal exposure, total glutathione (tGSH) and catalase (CAT) for oxidative capacity and malondialdehyde (MDA) for oxidative damage. In addition, cytosolic concentrations of several essential and non-essential (Ag, Cd, Cu, Pb and Zn) elements were determined. Biomarkers were measured spectrophotometrically, and element concentrations were quantified using a high-resolution inductively coupled plasma mass spectrometer. The MDA levels and the activity of CAT were significantly increased in the mussels from the contaminated site, indicating possible overproduction of reactive oxygen species and oxidative stress in these mussels. The elevated levels of these two biomarkers correlated with the increased concentrations of cytosolic Ag, Cu and Pb. Higher tGSH and MTs concentrations at the reference site coincided with higher cytosolic concentrations of Cd, a known inducer of MTs. Cytosolic Zn concentrations were similar in mussels from both sites of the Mrežnica River. The presented results suggest differences in biochemical responses of mussels from two sites with different pollutant loads. Therefore, the application of molecular biomarkers is a very useful tool for diagnosing the effects of environmental conditions on aquatic organisms.

1.08.P-Tu028 Life-Cycle Dietary Exposure to Polystyrene Microplastics on Medaka Fish (*Oryzias latipes*)

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The number of published studies evaluating the effects of microplastics (MPs) in fish has increased over the last two decades. However, there is paucity of information on how long-term exposures to secondary MPs may compromise overall fish development. In this study, 6-10 day-old post-hatch medaka (*Oryzias latipes*) fish were exposed to artificially degraded waterborne levels of 50 (i.e. 1X) and 500 (i.e. 10X) µg of polystyrene (PS) MP particles (200-µm range)/L for 150 days. These

concentrations corresponded to respective daily mean values of 247 and 3087 particles/L administered through the diet. The MP dietary exposure resulted in the presence of MP particles in body burdens. The biometric analyses found no incidence of MPs ingestion on fish growth and development. While the histological survey did not reveal severe alterations in potential target organs (i.e. gills and digestive tract), mild alterations in the 10X treatment were seen in other organs and included the kidneys and the thyroid gland. The initial days of the reproductive phase revealed MP-related differences in the number of gravid females, fecundity, and fertilization rates. Overall, these values reverted to normal rates over the succeeding days. The 150-day MP dietary exposure used in this study could not provide conclusive evidence of effects on medaka that could compromise overall growth/thriving and the ongoing reproduction.

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1.08 Understanding and linking effects of contaminants in wildlife across multiple biological levels (Virtual Only)

1.08.V-01 A Multi-Biomarker Approach to Evaluate the EcoToxicological Status of Common Kestrel Populations From Urban and Agricultural Environments

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The Common kestrel (*Falco tinnunculus*) is a small diurnal bird of prey included in the IUCN Red List as Least Concern due to its moderate but steady decline since the 1980s. Agricultural intensification, pesticide use, landscape modification that cause the reduction of foraging areas, and the availability of nesting sites have led to the decline of this species over time. Up today, very few studies evaluated the contaminants sublethal effects on common kestrel populations from areas with different anthropogenic impacts. This study aimed to evaluate the ecotoxicological status of *Falco tinnunculus* from anthropized areas, using a non-invasive multi-biomarker approach to obtain a diagnosis of environmental stressors effects. In 2020 and 2021, 68 blood samples from specimens of common kestrel nestlings were collected. The nest boxes of the animals are installed in an urban and an agricultural area of the Municipality of Rome (Italy) and in a control area. Neurotoxicity (BChE), genotoxicity (ENA assay, comet assay), immunotoxicity (differential WBCs count, SRBC hemolysis assay, bactericidal assay, NBT assay), and oxidative stress (TAS assay) biomarkers were evaluated. Some of these biomarkers were applied for the first time in this study. Moreover, the presence of parasites was also evaluated, and body condition was calculated for each nestling. The specimens from the agricultural area showed the highest level of DNA fragmentation, with statistically significant differences ($p < 0.05$) with respect to control and urban areas. BChE activity was significantly lower in birds from urban area with respect to agricultural area. The specimens from the urban area also showed the lower complement system activity while not showing a significant difference from the kestrels from the control and agricultural area. In conclusion, the kestrel nestlings of agricultural area showed the highest genotoxicity effects, otherwise the immunotoxicity effects were major in specimens from urban area. The results above will be integrated with the contaminant and population dynamics data to obtain more information on the interaction of common kestrel with the environment.

1.08.V-02 Chlorinated Paraffins, Diet and Endocrine Measures in Nestling Peregrine Falcons in the Canadian Great Lakes Basin

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Chlorinated paraffins (CPs) are complex mixtures of polychlorinated n-alkanes that are categorized as short-chain (SCCPs, C10-13), medium-chain (MCCPs, C14-17), or long-chain CPs (LCCPs, C?18). The differences in carbon chain length and chlorine content result in a wide range of physical-chemical properties that make them stable for a wide range of industrial applications, including lubricants, flame retardants, plasticizers, among other applications. They are produced in enormous quantities (2009: 1 M metric tons). In 2017, SCCPs were added to Annex A of the Stockholm Convention on Persistent Organic Pollutants. In Scandinavia, SCCPs, MCCPs and LCCPs were present in peregrine falcons (*Falco peregrinus*), with the highest concentrations found for LCCPs, which were the predominant CPs (55%). In the present study, we examine the CP profiles of nestling peregrine falcons sampled across the Laurentian Great Lakes Basin of Canada predominantly in 2017. Our objectives are 1) to compare and contrast exposure levels and profiles of CPs in peregrine falcon nestlings, 2) to determine any effects of diet (inferred from stable carbon, nitrogen and sulfur isotopes), biology (age, sex), and geography (region) on CPs and thyroid hormones, and 3) to examine possible relationships between CP exposure, circulating thyroid hormones, body condition, and size of the nestlings. As with the Scandinavian adult peregrine falcons, the peregrine nestlings in central Canada had circulating concentrations of vSCCPs (C?9), SCCPs, MCCPs that were dominated by LCCPs; Comparatively, the Canadian birds had lower concentrations of SCCPs, similar MCCPs, and higher levels of LCCPs (47%), likely reflecting differences in age and tissues sampled and potentially differences in exposure to CPs between Scandinavia and central Canada. Within southern Ontario, chicks raised within or to the east of Toronto (population: 4 million) had significantly higher concentrations of LCCPs (3245 ng/g lw) than elsewhere in Ontario. Circulating TT4 was significantly associated with vSCCPs ($N = 18$; $r = -0.62$ $p = 0.007$) and SCCPs ($N = 29$; $r = 0.38$ $p = 0.04$) overall. In the southern urban peregrine chicks, SCCPs were associated with estimated thyroid gland function consistent with previous findings involving the related American kestrel.

1.08.V-03 Development of a mRNA Biomarker Tool for Water Quality Monitoring With the Brown Trout *Salmo trutta*
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Input of pesticides, increased water temperature and pathogens represent a multi-stressor scenario common to many streams flowing through agricultural areas. This can negatively affect fish populations; however, effective methods to detect adverse effects in resident fish are limited. Gene expression biomarkers offer a promising effect-based approach to detect the sublethal effects of chronic exposures in the field. In this context, we aim to build a cost- and time-efficient tool for measuring a set of biomarkers (ca.100) by qPCR. For biomarker selection, we reviewed scientific literature and databases (e.g. AOP-Wiki) focusing on genes involved in specific modes of action as well as general stress responses. To identify further candidates, we carried out a transcriptomic analysis in brain and liver samples of juvenile brown trout exposed to a pesticide mixture (fluopyram, diuron, epoxiconazole, lambda-cyhalothrin and chlorpyrifos) for 14 days in the laboratory, at 12 or 15°C. We performed a second transcriptomic analysis on liver tissues of brown trout infected with *T. bryosalmonae*, the causative agent of the proliferative kidney disease (PKD). To integrate the molecular responses with potential consequences at higher levels of biological organization, we measured physiological health parameters (immune and energy metabolism, parasite infection success) and endpoints at the organism level (organ indices and growth). While no effect was detected on growth and immunoassays, respirometry measurements, reduced condition factor and hepatosomatic index indicated that pesticide exposure may negatively impact the energy status of brown trout. The transcriptomic results showed a massive effect of the 3-degree temperature difference on gene expression with 30 % and 15 % of transcripts being differentially expressed in the brain and liver (differentially expressed genes, DEGs). Pesticide exposure induced 1.26 and 2.29 % of DEGs in the brain, and 0.16 and 1 % of DEGs in the liver, at 12 and 15°C, respectively. The PKD disease caused 1.41% of DEGs in the liver mostly involved in immune pathways. Our results further highlight the importance of considering confounding effects of environmental parameters, such as temperature, when screening for biomarkers.

1.08.V-04 Metabolic Effect of Ocean Acidification on Common Cuttlefish *Sepia officinalis* Early Stages

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Atmospheric carbon dioxide (CO₂) emissions are continuously increasing due to the growing anthropogenic activities, causing a rise in the sea-surface partial pressure of CO₂ (pCO₂). This change in turn leads to decreased ocean pH, named ocean acidification, and affects the carbonate-silicate cycle. Such modification of seawater chemistry also affects the physiology and behaviour of marine organisms, impacting their metabolism, growth and development during vulnerable early-life stages. Among them, the embryo of the cephalopod cuttlefish develops for ~2 months) in encapsulated eggs with harsh conditions of hypoxia and hypercapnia, potentially worsen by the environmental ocean acidification. In this study, the development and the growth of early-life stages of *Sepia officinalis* were followed during the whole embryonic developmental period up to 10 days post-hatchling juveniles. Embryos and juveniles were exposed to five elevated pCO₂ conditions controlled with a continuous pH-stat system (pH 8.08; 7.82; 7.65; 7.54; 7.43). Metabolites were determined in ready-to-hatch embryos, just hatched embryos and 10 d-old juveniles, using a ¹H nuclear magnetic resonance (NMR) spectroscopy as a platform for untargeted metabolomics analysis. Consistent with previous studies, our results showed longer embryonic development and decreased hatching success at the lowest pH, but no effect on juvenile weight upon hatching. Metabolomics analysis revealed a metabolic depression in embryos reared at pH 7.43, non-monotonic changes to pH in 10 d-old juveniles, and no clear pH effect in newly hatched juvenile cuttlefish, likely due to the metabolic stress associated with hatching. Those results reveal possible effect of ocean acidification on the cuttlefish recruitment.

1.08.V-05 Microplastic Effects on Oxidative and Inflammatory Responses in the Japanese Quail

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Plastic pollution is currently recognized as a threat for wild birds. However, the effects of ingesting microplastics in birds are still not well understood. In particular, there are no studies thus far that have investigated the effects of smaller microplastics (< 1 mm). Oxidative stress and inflammatory responses to microplastic exposure have been reported in several species, like crustaceans, fish, and mice, but there is no such information in birds. Therefore, we exposed a laboratory bird model species, the Japanese quail (*Coturnix japonica*), to microplastics and investigated oxidative stress and biomarkers of inflammation (pro-inflammatory cytokines). The aim of this experiment was to compare the toxicity of two different sizes of microplastics, large microplastics (3 mm size) and small microplastics (< 125 µm size) of polypropylene and polyethylene in 55 growing Japanese quails. After 6 weeks of exposure, either to 3 mm particles (T1), powder < 125 µm (T2) or a mixture of both (T3), quails were euthanized, and blood was collected in heparinized tubes. Plasma and red blood cells (RBC) were separated and stored at -80 °C. Four antioxidant enzyme activities were analyzed in RBC samples, including catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and superoxide dismutase (SOD). Lipid peroxidation was estimated by thiobarbituric acid-reactive substances (TBARS) analysis. Plasma samples were used to analyze cytokine levels, including interleukin-1? (IL-1 ?) and tumor necrosis factor-alpha (TNF-?). Quails from T2 (powder) and T3 (mixture) treatment groups showed higher enzyme activities of CAT, GPx and GST than those in the control group (all *P* < 0.05). SOD activity and TBARS were not significantly different between treatment groups (*P* > 0.05). We did not find any significant differences between treatment groups in TNF-? and

IL-1 ? levels ($P > 0.05$). In this experiment, we provide evidence for the first time that small microplastic ingestion causes an increase of antioxidant enzyme activities in birds and we confirm that microplastic size is important for triggering sub-organismal responses.

1.08.V-06 Mitochondrial Texture of Humpback Whale Fibroblast Cells Upon Chemical Exposure

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The bioaccumulative, and often lipophilic nature of many environmental contaminants places cetaceans at a greater risk of accumulating elevated body burdens due to their longevity and high proportion of body fat. Ethical and logistical constraints prohibit *in vivo* toxicological investigation on cetaceans. An important focus of *in vitro* cetacean studies in recent years has involved cell culture. Image-based cell profiling using automated microscopy is a high-throughput and high-content strategy to analyse the effect of chemical exposure on cell morphology and function. Many chemicals and cell organelles can be assayed simultaneously. Its broad application in drug development, revealing subcellular sites of impact, renders it a potentially useful tool for the characterization of contaminant toxicodynamic. This study exposed both human (HF) and immortalised humpback whale (HuWa_{TERT}) skin fibroblast cell lines to 6 priority compounds (1,1-dichloro-2,2-bis(4-chlorophenyl) ethene, lindane, dieldrin, endosulfan, trifluralin and chlorpyrifos) known to accumulate in the Southern Ocean food web. Each cell line was exposed to the same concentration range of each chemical for visualization and quantification of mitochondrial texture effect using a range of stains. Analysis of image-based data on exposure of the cells to selected chemicals demonstrated concentration-, chemical- and species-dependent modulation of mitochondrial texture, with more significant changes observed in HuWa_{TERT} compared to HF. In addition, all 6 compounds were cytotoxic. We observed significant chemical-specific as well as concentration-specific inter-species sensitivity differences. These findings constitute the first fixed mitochondrial images of HuWa_{TERT}. Further, they provide unique insights into the potential toxicodynamics of these chemicals on mitochondria, the cellular ‘powerhouses’. Finally, the experiments reveal interesting insights as to relative inter-species sensitivity to selected compounds. Expansion of cetacean *in vitro* toxicity testing is needed for evidence-based conservation and management action.

1.08.V-07 Monitoring the Effects of Contaminants at Different Levels of Biological Organization in Great Tit (*Parus Major*, Linnaeus, 1758) From Rural and Urban Areas of Northern Italy

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Pesticides, widely used in agriculture, and other classes of anthropogenic contaminants, can cause reversible alterations or permanent damage to biota, including avian species. Great tit (*Parus major*), the species selected for this research, is ubiquitous, has a good ecological relevance and its territoriality can provide precise information on a given territory due to no variables deriving from migration. Starting from an original idea of Matteo Griggio, in the last four years several nest boxes for great tit, were installed in several areas of Veneto region (northern Italy), characterised by different anthropogenic impact. This work aimed to develop and apply a non-invasive method to monitor, in the selected species, the effects of anthropogenic contaminants at different levels of biological organization. We explored potential interlinkages between effects at molecular and cellular level and effects at behavioural, phenotypic and population level. Nestlings great tit were sampled in urban, agricultural, and wooded environments. We developed and applied a non-invasive protocol on blood and excreta, to test different toxicological responses. Neurotoxicity (esterases inhibition), genotoxicity (ENA assay, comet assay), immunotoxicity (complement system), estrogenic (vitellogenin) and oxidative stress (TAS assay) effects were evaluated in the specimens from the different areas. At higher biological levels, parental care (nest defence and pair coordination over offspring provisioning) of great tits breeding, and cognitive abilities, by problem solving tests, as well as phenotypic endpoints and reproductive success were assessed on the target species. Responses to contaminants at different biological levels were modulated depending on the characteristics of the study areas and interlinkages between responses at different biological levels were found. A strong increase of the total antioxidant system was found in Great tit from agricultural areas, as well as genotoxic effects (Comet assay), present also in urban areas. Body condition index was found altered in the agricultural area. Responses of handling stress, such as tonic immobility showed differences between urban and agricultural sites, while breath rate was affected in urban area. The developed protocol was proved to be a useful tool for the ecotoxicological monitoring of great tit. An integrated approach is fundamental to obtain a complete picture of the health status of the studied species. *Dedicated to the memory of Matteo Griggio.*

1.08.V-08 One Health: Are SARS CoV-2 and Some Chemicals Synergistic?

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Although the relationship between environmental toxicity and infectious diseases is little studied, it is likely to be more common than is recognized. For instance, some conditions that are known to influence COVID-19 progression could have toxicological causes such as cancer, endocrine, neural and inflammatory disruptions, renal failure, etc. Very early in the pandemic it was noticed that COVID-19 patients from countries/regions with the highest air pollution presented more severe clinical symptoms. As a consequence of the quarantine imposed by the high mortality associated with COVID-19, a significant decrease in air pollution was reported resulting in a reduction of SARS-CoV-2 adverse effects. This contribution is highlighting that SARS-CoV-2 and some high production volume chemicals like Ni, Cd, atrazine, 2,4-D and Bisphenol A could exert dysfunctions on HIF-1 and ACE2 with a potential synergistic effect on COVID-19 severity. By means of early life stages toxicity studies (e.g. AMPHITOX),

among the multiple terato and dysfunction effects, some chemicals like Bisphenol A, 2,4-D and atrazine could produce osmoregulatory disruption resulting in hydropsy that could be associated to ACE2 receptor expressed in early embryonic stages. ACE2 was associated to intra-alveolar edema and is the main multiorgan entrance point of SARS-CoV-2. It is noteworthy that hydropsy was reported in COVID-19 fetus. The same chemicals plus Ni and Cd could be related to COVID-19 severity by means of their effect on oxygen consumption. It is well documented that hypoxia triggers the hypoxia inducible factor (HIF) and hypoxia associated with COVID-19 could implies an over-activation of the HIF system influencing the functionality of 200 genes, including those that regulate apoptosis. It is worth mentioning that the risk of toxicity increases with age which implies higher bioaccumulation and biomagnification of toxics, potentially contributing with the most severe clinical symptoms of COVID-19, including high mortality of the aged people. There is an increasing interest to conduct integrated risk assessment combining the processes of risk estimation for humans, biota and natural resources. Early life stage vertebrate embryos as sentinel organisms with morphological and functional end points for toxicity could represent an optimization toward a holistic approach for toxics control to avoid potential synergistic effect of chemicals with infectious agents.

Track 2: Ecotoxicology becomes stress ecology: From populations to ecosystems and landscapes

2.01 Aquatic and terrestrial plant ecology, ecotoxicology and risk assessment (Part I)

2.01.T-01 Cationic and Anionic Silica Nanoparticles Reduce the Toxicity of the Pesticides Pentachlorophenol, Paraquat and Diflufenican to Algae

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Colloidal silica shows promising properties as efficient adsorbents for pollutants in water and hence reducing the pollutant's bioavailability to aquatic organisms. To explore this, the present study investigated the toxicity of pesticides in mixtures with colloidal silica towards the freshwater green algae *Raphidocelis subcapitata*. Three spherical silica nanomaterials with different surface charge, weakly anionic, strongly anionic and cationic, were tested in binary mixtures with three differently charged pesticides: cationic paraquat, anionic pentachlorophenol and neutral diflufenican. The hypothesis was that the silica nanomaterials would bind oppositely charged pesticides and hence reduce the toxicity to algae. To test this theory, different nanomaterial concentrations (0.4-50 mg/L) were tested in mixtures with a fixed concentration of the pesticide known to reduce algae growth. The results showed that the strongly anionic silica was able to reduce the growth inhibition of paraquat with up to 60%. The cationic nanomaterial reduced growth inhibition with 30-50 %. The cationic material also reduced the toxicity of diflufenican by 10-20 %. Adsorption of pesticides onto the various nanomaterials was further confirmed by chemical analysis with LC-MS. To conclude, colloidal silica were efficient adsorbents of pesticides, which led to a reduced bioavailability and toxicity to algae.

2.01.T-02 Ecotoxicity, Adaptation and Gene Expression of the Green Alga *Raphidocelis subcapitata* Towards the Herbicide Diflufenican

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Research on algal ecotoxicology towards herbicides has been mainly focused on short-term responses. However, the development of tolerance via physiological and genetic changes that allows algae to adapt in an ecological time scale to toxic substances, remains poorly understood. This study aims to describe the ecotoxicity and adaptation mechanisms of the freshwater green microalga *Raphidocelis subcapitata* to the herbicide diflufenican, a carotenogenesis inhibitor commonly found in European agricultural streams. To do so, an adaptive laboratory experiment was performed for 90 days. *R. subcapitata* was exposed to three different concentrations of diflufenican with environmental and ecotoxicological relevance (i.e. 0, 10.7 and 310.5 ng/L). During the first week of exposure, a decrease in growth rate up to 55.5%, carotenoid and xanthophylls pigments up to 38.7%, and PSII photosynthetic activity up to 17%, indicated that diflufenican had a negative effect on all these three physiological endpoints. This stress phase was followed by a recovery and an adaptation phase, during which the apical endpoints of the exposed alga returned to similar values compared to their controls. Diflufenican-adapted alga developed tolerance to diflufenican exposure (higher EC50s). However, the adapted alga did not show differences in fatty acids composition, indicating that changes in the lipid composition of the cell membrane were not involved in adaptation processes. *R. subcapitata* showed a removal rate capacity of 10% both at the stress and adaptation phases, suggesting that adaptation mechanisms are not linked with an increase in biodegradation capacity. Our findings reflect the high sensitivity of algae to diflufenican and its ability to adapt, as well as the importance of investigating the effects of herbicides after long-term exposures. Finally, a planned transcriptomic analysis will potentially highlight the genes and enzymes that play a role in bioaccumulation, detoxification and overall adaptation processes that allow the cells to survive under different concentrations of diflufenican (evolutionary ecotoxicology), as well as their implications in ecological functions.

2.01.T-03 Effects of Environmental Parameters and Toxicants on *Lemna minor* Growth: An Integrated Experimental and Modelling Approach

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Living organisms can be exposed to radionuclides and heavy metals. Large areas, including surface waters, are contaminated with pollutants and in need for remediation. Phytotechnologies use biological processes of living plants to remove, degrade or stabilise pollutants in environmental compartments such as soil, water and air. Phytoremediation is already known as an efficient site remediation technology for various types of pollutants and can be used as a complementary waste water treatment technique for specific scenarios. *Lemna minor*, commonly known as duckweed, has the possibility to absorb and accumulate pollutants in its biomass and can therefore be used for remediation of contaminated water. It is important to understand the behaviour of *L. minor* to remove pollutants from the aquatic environment, assessing its remediation capability and proposing proper remediation strategies. A mathematical model for *L. minor* growth and transfer of pollutants can help to evaluate and select phytoremediation as appropriate remediation strategy for specific scenarios. Building on a previous growth model, a more comprehensive growth model is developed by incorporating the impact of additional environmental conditions using an experimental and modelling approach. To study growth changes of *L. minor*, experiments are performed under different environmental conditions where temperature, light intensity, photoperiod and nutrient compositions are varied from optimal to very low or high conditions. For each experimental condition, growth curves are created and used to optimise these environmental parameters in the growth model. After an overall optimisation and validation of the global growth model, the model will serve as a basis for the remediation model, where new insights on uptake of radionuclides and other pollutants can be incorporated. For several (mixtures of) pollutants, uptake and dose response curves are being made as well as time-dependent uptake and release curves. In addition, experiments that study the effects of pollutants on photosynthesis and pigment, starch and soluble sugar content of *L. minor* are also performed. Together with the research for the development and optimisation of the growth model, these first (non-published) pollutant results will be presented at the conference. Finally, when the outcome of the *Lemna* based system can be model-predicted, it can be applied as an efficient, cost-effective and eco-friendly water depollution technique.

2.01.T-04 Toxicogenomic Analysis of Ecotoxic Modes of Action in *Lemna minor*

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Due to its high reproduction rate, *Lemna minor* is one of the most frequently used aquatic model organisms in ecotoxicology. As a primary producer, it is an important part of the ecosystem and therefore an indicator for water quality. If the development of the plant is influenced by external factors such as xenobiotics, this will not only have a direct effect on the phenotype of the plant, but also indirectly affect other organisms. Therefore, *L. minor* is commonly used for the evaluation of toxic effects on water plants by newly developed substances such as pharmaceuticals or pesticides. In this study, *L. minor* was used for investigations of ecotoxic modes-of-action (MoA) at the molecular level. Therefore transcriptomics and proteomics methods were applied to a shortened version of the OECD guideline test No. 221 conducted with low effect concentrations of two model substances – the pharmaceutical atorvastatin and the herbicide bentazon. Previous studies have shown that, besides photosynthesis inhibition, bentazon additionally has a similar MoA as atorvastatin, whose action is based on the inhibition of the key enzyme of cholesterol and phytosterol synthesis 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR). The goal of our study was to link the determined molecular fingerprint of each substance with both its hazardous effects on the test organism and its MoA in order to identify possible biomarkers. Therefore, we applied bioinformatics approaches to functionally annotate the previously unannotated reference genome of *L. minor*. Our functional annotation pipeline is in principle applicable to any organism with an available reference genome and thus greatly facilitates the identification of gene functions for poorly annotated organisms. For both test substances, significant changes in gene expression were observed in the context of a shortened ecotoxicity test with low effect concentrations. These molecular changes preceded the physiological effects and may, therefore, be consulted for early predictions.

2.01.T-05 Selecting and Testing Ecologically Relevant Macrophytes and Endpoints in Mesocosm Studies

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Aquatic macrophytes play an ecologically important role in the ecosystem by providing shelter, food and performing essential biological and ecological functions. Aquatic freshwater mesocosms are designed to represent edge of field water bodies by creating a habitat that will support communities of aquatic macrophytes, algae, zooplankton and macroinvertebrates in a contained system. Therefore the macrophytes included in these systems should reflect the communities found in the natural environment. The EFSA guidance document on tiered risk assessment for plant protection products (PPP) for aquatic organisms in edge-of-field surface waters, makes recommendations for a minimum of 8 sensitive/vulnerable taxa with acceptable minimum detectable difference (MDD) values to be tested. However there is little guidance on selecting representative macrophytes that will survive in these systems, or on the selection of appropriate endpoints that will provide regulatory acceptable data. In addition, the limited number of standard macrophyte laboratory tests makes it challenging to determine which species may be sensitive to any given stressor. The aim of this presentation is to share our expertise on the selection of macrophyte species and techniques for assessing plant growth and health in mesocosm studies. We hope that by sharing our considerations for macrophyte selection and endpoint evaluation in mesocosm studies we can open up the discussion on the relevance of mesocosm studies, particularly within the future of risk assessment for macrophyte communities. We will also demonstrate the CEA sloped mesocosm design as a reference study in order to derive more reliable and robust population and community level endpoints using macrophytes as an example. These results can be used to inform the experimental design of future macrophyte mesocosm studies such that the results meet the EFSA requirements for sensitive/vulnerable taxa with acceptable MDD values for regulatory use.

2.01 Aquatic and terrestrial plant ecology, ecotoxicology and risk assessment (Part II)

2.01.T-06 Combined Impact of Agricultural Run-Off and Warming on Primary Producers and Consumers in Agricultural Streams

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Ditches or small streams in agricultural landscapes are among the first to be affected by agricultural run-off (ARO) composed of nutrients and pesticides. In artificial streams, we investigated the effects of warming and ARO on the temporal dynamics of different types of primary producers (macrophytes, periphyton, phytoplankton, filamentous blanketing algae) and primary consumers. Our hypotheses were that warming would favour early macrophyte development and that ARO would favour filamentous algae over macrophytes in these systems. The experiment was based on a 2 x 2 factorial-design (warming, ARO) with each 4 replicates. Channels had an upstream compartment containing *Glyceria maxima* and *Dreissena polymorpha*. The downstream channels were planted with root or rhizome sections of three submerged macrophytes, received an inoculum of periphyton, alder leaf litter, *Gammarus roeseli* and *Lymnaea stagnalis*. The effect of ARO on primary producers was very distinct. ARO significantly enhanced the development of blanketing filamentous algae and periphyton, while warming had only minor effects. The high dose of nitrate applied with ARO vanished very fast, probably absorbed by filamentous algae and periphyton. When filamentous algae collapsed towards the end of the experiment, phytoplankton increased, probably triggered by a massive liberation of phosphorus. Macrophytes developed very late in the cold spring in 2021. *Potamogeton perfoliatus* dominated, profiting most from warming. ARO negatively affected its development at a later stage in the experiment, suggesting indirect effects caused by competition with blanketing filamentous algae. All other macrophytes showed no clear response to warming or ARO. ARO had a strong effect on the reproduction of gammarids and snails, likely caused by pesticides and leading to a reduced periphyton control. ARO and warming strongly enhanced mortality of mussels. Leaf litter degradation was enhanced by warming, but not affected by ARO. Submerged macrophytes usually appear only after the water temperature reaches a certain temperature, and often have to compete against a massive development of blanketing algae. Our study aims to determine whether ARO affected this dynamic. Combined nitrate and pesticide effects led to a range of direct and indirect effects on primary producers and consumers, with the most visible being the strong development of filamentous green algae, but also effects on periphyton, gammarids and snails.

2.01.T-07 Uptake, Translocation, and Metabolism of Different Contaminants of Emerging Concern in Radish Crops Grown Under Controlled Conditions

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The constant search for good quality water for agriculture has meant that water resources are under pressure in many regions, representing the main limiting factor for social welfare and economic development of many countries. Treated wastewater represents a valid alternative to the consumption of good quality water for the irrigation of agricultural crops. However, this practice could contribute to the spread of some organic contaminants in the soil and in the environment. In fact, the presence of contaminants of emerging concern (CECs) is notoriously reported in effluents of wastewater treatment plants (WWTPs), since conventional treatments are not specifically designed for their removal. Their impact is of particular relevance for the disposal and re-use of wastewater in agriculture due to the use and accumulation of CECs in food crops and the consequent spread in the food chain and ecosystems. Radish (*Raphanus sativus*) is often used as a model plant to investigate the fate of organic contaminants in plants given its short growing cycle and its potential ability to accumulate contaminants in the roots. In fact, the contaminants could enter the plants from the soil through the absorption of the roots, and constitute a potential risk to food safety. In addition, to accumulate in the root, these compounds can give rise to phenomena of translocation in the leaf parts or potential metabolism with the formation of a variety of transformation products. However, only a few studies were designed to evaluate the uptake and the potential metabolism of pharmaceuticals in radish and only for a limited range of compounds [1, 2]. This study was therefore planned to evaluate the influence of a wide range of pharmaceutical compounds into radish crops irrigated with artificially contaminated water at 10 and 100 µg L⁻¹. In addition to uptake and translocation, the metabolic transformation of pharmaceuticals was also evaluated. Pharmaceuticals in radish roots and leaf material were extracted by one-step modified QuEChERS method [3], using whereas the detection was performed using the new SCIEX X500R QTOF-MS, a compact hybrid quadrupole time-of-flight mass spectrometer combining advantages of TOF and QqQ systems with accurate mass. In this study, we investigated the use of MRMHR and MS/MS^{All} with SWATH acquisition for the targeted analysis (using reference standards) and non-target screening on a routine basis.

2.01.T-08 Comparative Transcriptomic Analysis of Brassica Napus L. Grown on Soils Contaminated by Metallurgical Industry Reveals New Insights Into PLANTS' Response to Metal Stress

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Problem with heavy metal-contaminated soils is increasing significantly throughout the world due to industrialization, mining

operations, improper waste, and water treatment. Although the response of plants to single metal contamination in lab condition are extensively researched, the aspect of mixed toxicity, which usually occurs in the natural world, is often overlooked. Our knowledge and understanding of the specific response to synergistic stressors on plants are strictly limited. The main aim of the experiment was to assess the transcriptomic changes in leaves of *Brassica napus* after exposure to soil contaminated with a vast range of heavy metals due to the past metallurgical activity in the area. Specifically, the contaminated soil contained over 20 mg/kg of cadmium (Cd), over 1100 mg/kg of lead (Pb), and over 700 mg/kg of Zinc (Zn). Thus, the purpose was to identify how such actual industrial contamination influences plants on the transcriptome level to broaden our understanding of the actual environmental impact. The experiment was performed in actual field conditions on 6 plots and lasted for the entire vegetative season. All the cDNA libraries of *B. napus* were sequenced using an Illumina HiSeq™ 4000 platform. The sequencing generated 21 688 894 to 29 810 359 raw paired reads for each sample. The soil supplementation with sewage sludge caused 10 025 genes to be differentially expressed with the False Detection Threshold at a level of 0.001. The gene ontology analysis showed that most differentially expressed genes were related, among others, to plants' response to stress, cell death, xenobiotics, hormone production, and transport. The project consisted of a first study dealing with the influence of long-term exposure to mixed metal toxicity application on plant metabolism on a transcriptomic level. *Acknowledgement* - Research was funded by National Science Centre, Poland, 2018/31/N/NZ9/01742

2.01.T-09 Drifting Towards HIGHER Tiers: Developing a Regulatory Greenhouse Test Method for Non-Target Terrestrial PLANTS Mimicking Realistic Drift Exposure to Refine the Risk Assessment of Herbicides

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The risk assessment for non-target terrestrial plants (NTTPs) in Europe rests on two pillars: endpoints derived of standard guideline tests and exposure estimations derived from Rautmann drift value tables. Both pillars have been challenged in recent years. Endpoints are criticized as being not realistic enough while drift values will be replaced by more conservative drift values (SETAC DRAW). Accordingly, a more realistic higher tier approach leading to a simultaneous refinement of effects and exposure would be desirable. One possibility could be a recently developed field study design assessing effects of a herbicide on plants after exposure via drift [Moore et al. 2021. Integr Environ Assess Manag]. As the approach is time-, labour- and cost-intensive, a laboratory Plant Intermediate Tier Test (PITT) design is proposed. This study design will follow the guideline protocol for NTTP greenhouse tests but exposure will mimic drift such as the plants would experience in the field. Here, we present the spray cabin set-up as well as results of modelling and measurements used for calibration of the spray cabinet. Spray cabinets will be adapted as follows: (a) volume lowered, (b) use spinning disc nozzle in order to generate a droplet spectrum similar to that of a drift plume, and (c) place plants on a conveyer belt moving them through the spray cloud so that the relative movement of the plant and spray cloud are similar to that of a stationary plant in the field and a drifting spray plume. The SiMoD boom sprayer model was used to predict the quantity and droplet spectrum of drift reaching NTTPs in order to determine how the spray cabinet needs to be calibrated when performing a PITT trial. The quantity of spray deposited is expected to correlate with the quantity of airborne spray. The main factors influencing the droplet size are the wind speed and distance downwind. Interestingly, nozzle design has a very small effect on downwind droplet size distributions. The proposed Plant Intermediate Tier Test (PITT) method could serve as a refinement option for the NTTP risk assessment, refining exposure and effects at the same time while still being labor-, cost- and time-efficient.

2.01 Aquatic and terrestrial plant ecology, ecotoxicology and risk assessment (Virtual Only)

2.01.V-01 Algal Preservation and Enumeration Techniques for Use in Micro and Mesocosm Studies

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Standard, lower tier, testing strategies for the effects of Plant Protection Products (PPP's) on algae are focused on single species studies and may not provide adequate protection for these important communities. Higher tier approaches, such as micro/mesocosm studies are designed to represent edge of field water bodies by creating a habitat that will support communities of aquatic macrophytes, algae, zooplankton and macroinvertebrates in a contained system. Therefore, these experimental systems offer a more comprehensive analysis of the effects of PPPs on these communities. Whilst definitively more representative of natural systems, this increase in complexity brings further challenges. Freshwater algal communities are very diverse, the taxonomic identification of these species is difficult, and requires trained taxonomists. When designing these studies, consideration needs to be given to the methods employed for sampling, preservation and enumeration to ensure all steps are fit for purpose. The sampling processes need to offer a true representation of the algal assemblages present. One of the major difficulties in sampling freshwater algae is preservation. The need for preservation at all should be considered as they will damage algal morphology over time, however this step is often a necessity in larger, long-term studies. Following sample collection, enumeration is necessary to collect the data needed to derive ecotoxicological endpoints, e.g. MDD's, EC_x and NOEC's. As with the sampling procedures, this process needs to use sub-samples that accurately reflect the whole test system. Here we present and assess different methods that can be used for the sampling preservation and enumeration of freshwater phytoplankton and periphyton. We discuss the pro's and cons of these approaches and present methods for the analysis of microcosm and mesocosm algal samples that have provided the most reliable and robust statistical data from studies conducted at CEA.

2.01.V-02 Assessing the Acute Toxicity of Individual Polycyclic Aromatic Compounds to Furoid Macroalgae (*Fucus vesiculosus*) Using a Germination Assay

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Of the 1000s of components within crude oil, polycyclic aromatic compounds (PACs) are thought to be the component of crude oil primarily responsible for toxicity. Due to the complexity of a crude oil spill, stakeholders must rely on mathematical models to predict and assess the impacts of a spill on aquatic life. Oil spill models rely on quantitative structure-activity relationships (QSARs) like the target lipid model (TLM) to estimate the toxicity of different components found within crude oil, as well as species sensitivity distributions (SSDs) to determine species at risk if exposed. Macroalgae play a critical role in both freshwater and marine ecosystems and function as both primary producers and as a habitat for other aquatic life, however very little research has been done to determine the sensitivity of macroalgae to PAC exposure. The aim of the study was to generate toxicity data to determine the sensitivity of *Fucus vesiculosus*, a common intertidal brown seaweed, to PACs, and to assess the impacts of different environmental conditions on observed toxicity. Test solutions were generated using passive dosing with polydimethylsiloxane (PDMS) O-rings left to equilibrate in glass petri-dishes for 24-48 hours. To generate zygotes for the germination assay, the receptacles of sexually mature plants were dissected, sexed, and stored in the dark at 4°C until use. Using previously published spawning protocols, zygotes were generated and allocated to glass slides submerged within the glass petri-dishes. Germination success was assessed at 3 and 5 dpf. Of the compounds tested and assessed at 3 dpf, dibenzothiophene had the most significant impact on germination rate ($EC_{50} = 1.59 \mu\text{mol/L}$), followed by naphthalene, and methylnaphthalene ($EC_{50} = 5.46 \mu\text{mol/L}$, $EC_{50} = 8.07 \mu\text{mol/L}$ respectively). There was a linear relationship between the log (LC_{50}) and log (K_{ow}) of the PACs as described in the TLM, and the critical target lipid body burden (CTLBB) was determined to be $29.1 \pm 12.4 \mu\text{mol/g}_{\text{octanol}}$. The results from this study suggests the zygotes of *F. vesiculosus* are one of the more sensitive species and life stages included in the TLM model and would serve as a good model species to be included in crude oil toxicity tests. The data generated in this study can be used to better understand the sensitivity of macroalgae to PAC exposure, and to assess the impact of shoreline oiling on the germination success of *Fucus* algae.

2.01.V-03 Effects of Tire Fragments Toxicity on the *Vigna radiata*

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Tire wear particles, generated from the roads, is one of the main sources of microplastics in the terrestrial environment. This study evaluated the effects of tire particles on mung bean (*Vigna radiata*). Three different types of tire particles (bicycle (B), car (C), and electric scooter (ES)) were exposed to mung bean for 4 weeks and the growth inhibition were measured. Additionally, the tire fragments were leachate for 4 weeks to analyzing the toxicant leaching from the tire fragments. There was some shoot growth inhibition on the exposure group of ES tire fragments. The adverse effects of tire fragments were expected to be induced by the chemical leaching from the tire particles, that the ICP-MS and non-target GC-MS were conducted on three types of leachates to figure out the major toxicants. According to the chemical analysis the tire leachates contained the high concentrations of zinc and benzothiazole in all three leachates, which were used as the additives of plastic-products. Especially in ES leachate, the chrome and lead were detected and showed the dominant correlation with the plant toxicity. Also, there was some size difference between the tire fragments and the ES tire fragments have the small size compared to the others. This finding of this study suggests that tire fragments from the land release the toxic components and the size of tire particles could also induce the plant toxicity.

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2.01.V-04 Transfer and Bioaccumulation of Lead and Zinc in Different Tissues of Blackberry

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Plants can accumulate heavy metals from soils, air, and water, which makes them suitable biomonitors. Plant biomonitoring is therefore a useful tool for geochemical risk assessment. Certain plants can also be used for phytoremediation of polluted soils. This study was conducted along a river system (Innerste River) to determine soil-to-plant bioconcentration factors (BCF) and translocation factors (TF) for lead and zinc in naturally occurring blackberries (*Rubus fruticosus* L. agg.). Additionally, we investigated whether heavy metal concentrations in floodplain soils and leaves of blackberries growing in the floodplain and in adjacent regions outside the floodplain decrease with distance from the source region of contamination. The root system of blackberries enters deeply into the soil (some > 1 m), which makes the species suitable as a biomonitor of soil contamination. Consumption of plant parts with an elevated metal content may pose a health risk for humans and animals. As a result of historical mining, processing and smelting processes of metalliferous ores in the Harz Mountains and the associated runoff from tailings and slag heaps, large amounts of heavy metals (Cd, Pb, Zn and Cu) were and still are fluviually transported into the Innerste floodplain. Hence, river sediments and floodplain soils in the immediate vicinity of the point sources as well as floodplains further downstream are heavily contaminated with heavy metals. The soil heavy metal concentration, in the regularly flooded areas (mean of 1457.22 mg Pb/kg and 1365.15 mg Zn/kg), far exceeds the precautionary values of the Federal Soil Protection and Contaminated Sites Ordinance applicable for Germany. It was determined that the concentrations of Pb as well as Zn in the roots and leaves were proportional to the soil heavy metal content. Both studied soil and plant heavy metals differed significantly between the floodplain and its adjacent non-flooded area, while pH and soil organic matter showed no significant difference. With the exception of BCF(Root) Pb, BCFs and TFs were found to decrease exponentially with soil and root heavy metal concentrations. BCF was < 1.0 for both heavy metals and TF was < 1.0 for Pb, but > 1.0 for Zn. Our results indicate that leaves

and roots of blackberry can be used as indicators of soil contamination by Pb and Zn, but that the species is inappropriate for phytoremediation.

2.01.V-05 UV Radiation Modulates the Combined Effects of Depleted Uranium and Gamma Radiation on Aquatic Macrophyte *Lemna minor*

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Elevated concentration of background ionising radiation and radionuclides in the aquatic environment have been observed especially in radioactively contaminated areas such as Chernobyl (Ukraine), Mayak (Russia), Fukushima (Japan) and NORM sites such as mining in Kazakhstan and Canada, and pose a threat to aquatic ecosystems. In addition to ionising radiation and chemical stress from the radionuclides, UV radiation, a common ubiquitous environmental stressor is expected to act as a co-stressor on aquatic organisms inhabiting the upper water layers. All these stressors may interact with each other and cause combined effects that are challenging to predict. The aim of this study was to characterize the combined effects of depleted uranium (DU), ionizing radiation (γ -radiation) and UV radiation on the aquatic macrophyte *Lemna minor*. In the presented study, the aquatic macrophyte *Lemna minor* was used as the model species to assess and exposed to different level of γ -radiation (10-40 mGy h⁻¹), DU (DU ((UO₂(NO₃)₃, 0.2-20 mg L⁻¹) and a fixed UVB radiation (0.5 W m⁻²), either alone or in combination for 7 days. After exposure, the reproduction of *L. minor* was measured to characterize the adverse effects of these stressors at individual level, while the responses of oxidative stress (ROS formation), photosynthetic performance (PSII efficiency) and mitochondrial dysfunction (mitochondrial membrane potential, MMP) were investigated at subcellular and cellular levels. The combined effects were evaluated using a three-way analysis of variance (3W-ANOVA) and a modified Independent Action (IA) model. The results indicated in binary exposure, combination of UVB and γ -radiation induced synergetic inhibition on reproduction, while antagonistic and additive effects were observed in most endpoints at subcellular and cellular level, such as ROS formation, PSII performance and MMP. The combined exposure of DU and high dose rates of γ -radiation (40 mGy h⁻¹) caused significant interactions consistent with synergism on the reproduction of *L. minor*. In ternary exposure, UVB radiation significantly interact the combined effects of DU and γ -radiation on reproduction at high dose rates of γ -radiation (?20mGy h⁻¹). This study demonstrated that γ -radiation, DU and UVB radiation cause combined effects that were both target-specific and concentration (dose rate)-dependent. No interaction and additivity was identified to be the most common effect, whereas synergistic interactions were mainly observed at high levels of γ -radiation and DU. It is anticipated that these results will contribute to inform cumulative hazard and risk assessment for multiple stressors such as ionising radiation and chemical stressors. Additional endpoints and statistical models will be implemented to provide the mechanistic understanding and analytical strategies to identify the most relevant toxicity pathways for the single and multiple stressors tested.

2.01 Aquatic and terrestrial plant ecology, ecotoxicology and risk assessment (Poster)

2.01.P-Tu029 Cigarette Butts Leachates Produce Toxic Effects at the Biochemical and Population Levels in the GREEN Microalgae *Pseudokirchneriella subcapitata*

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Cigarette butts are polluting residues resulting from tobacco smoking, which are present everywhere. It has been estimated that 4.95 billion cigar butts are discarded annually throughout the world. In urban areas, butts are often discarded on the street or anywhere, without any restrictions, so they can accumulate on the ground and can be leached and reach aquatic environments through drainage and sewage system. Microalgae have been used as bioindicators because of their sensitivity to pollutants and their importance as primary producers in aquatic communities. In this study, the toxic effects of leachates from cigarette butts in the green microalgae *Pseudokirchneriella subcapitata* were assessed. Population growth, the concentration of photosynthetic pigments, and the content of proteins, lipids, and carbohydrates were quantified in chronic exposures. The cigarette butts were collected in hermetic containers from voluntary smokers and were selected to study only those of one brand (the most popular); the remains in the butts were manually eliminated. To prepare the extract, 120 butts were placed in 1 L of deionized water and stirred for two hours at room temperature; the leachate was vacuum filtered using autoclaved 0.22 μ m mesh size membranes, under axenic conditions. To determine the 96-h Mean Inhibitory Concentration (IC₅₀), *P. subcapitata* was exposed to the leachate containing the equivalent to 0.012, 0.12, 1.2, 12, and 120 butts L⁻¹, at 25 ° C and continuous illumination. The determined IC₅₀ was 76.9 mg of cigarette butt L⁻¹, equivalent to 0.4 butts L⁻¹. Although toxic inhibitory effects were observed in the population growth of *P. subcapitata*, no significant effects were detected in the content of photosynthetic pigments or the protein concentration. However, a significant decrease in carbohydrate and lipid content was documented. The undiluted leachate caused complete inhibition of growth of *P. subcapitata* since the first exposure hours. Leachate from butts was very toxic to this microalgae, so it is essential to study the effect of this ubiquitous waste due to the environmental consequences in aquatic biota. It is also necessary to regulate the disposal of these wastes to the environment to avoid or reduce biological impacts.

2.01.P-Tu030 A Microwell Plate Based Bioassay for Toxicity Testing Using the Benthic Desmid Species *Closterium ehrenbergii*

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Closterium ehrenbergii is a large unicellular benthic green alga belonging to the class Zygnematophyceae, which represents the closest relatives of land plants. It lives not free floating but attached to filamentous algae and macrophytes in freshwater. With its extraordinary cell dimensions of up to 800 x 100 µm *C. ehrenbergii* is visible with the eye and ideally suited to be observed under little magnification using a simple stereomicroscope. This property enables the use of *C. ehrenbergii* in a miniaturized bioassay for ecotoxicity testing using microwell plates and the absolute cell number as end point without being dependant on surrogate parameters such as absorbance or chlorophyll fluorescence. Cells are transferred in small groups of 10 ± 2 individuals by micropipette to each well of a 24 microwell plate. Subsequently 1 ml of culture medium including the dissolved test substance or pure medium for controls respectively is introduced to the wells. A number of 6 replicates per concentration step ensures high statistical power with variation coefficients of 8 – 12 % on average. The number of cells in every well is counted manually using 10x magnification every 24 hours. No counting chamber is needed since the total amount of cells per sample is recorded. Growth rate is determined from the logarithmic growth function $\mu = \ln(x_t/x_0)/t$. In accordance with OECD 201, test duration is 3 days, whereby illumination may not be continuous since cell division takes place during the dark period. Hence, a light-dark regime of 14:10 h is favourable. Also, light intensity should not exceed $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ to avoid cell damage. C-medium is most suitable for cultivation. Figure 1 shows the results of an ecotoxicity test with *C. ehrenbergii* and 3,5-dichlorophenol as a reference toxin for validation of the new test system: Fig. 1. Dose response curve showing inhibition of growth rate against concentration of 3,5-DCP. The approach of miniaturization for ecotoxicity tests has several advantages: 1. *Closterium ehrenbergii* represents a benthic charophycean alga which has not been standardized in this way yet and is an addition to the chlorophycean planktonic species listed in OECD 201. . 2. The number of living organisms which are sacrificed by a test is dramatically reduced.. 3. The amount of all kinds of chemicals such as toxicants as well as nutrients is reduced to a minimum. . 4. Due to a higher number of replicates without increasing the experimental effort in comparison to traditional test designs using flasks or test tubes, the statistical power is increased. Collaboration between Hochschule RheinMain, university of applied sciences and Georg-August-university Göttingen. Co-authors: PhD László Dören, Hochschule RheinMain; PhD Thomas Friedl, university of Göttingen; PhD Antje Gutowski, AlgaLab.

2.01.P-Tu031 Exploring Modes of Action and Concentration-Response Patterns in the High Throughput 96-Well Algae Growth Assay

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The 96 well-plate algal toxicity assay with the green algae *Raphidocelis subcapitata* has been increasingly used for assessing toxicity of samples with complex mixtures of chemicals. This is traditionally done by measuring algae cell density over 48 hours by measuring absorbance at 684 nm, or fluorescence at 440 nm (excitation) and 680 nm (emission). However, we have observed that some water samples with complex chemical mixtures often lead to largely different absorbance and fluorescence response patterns and biphasic concentration-response curves. This raises questions regarding the choice of method and measure, but also leads to questions on which chemicals could be driving the different response mechanisms. To address this issue, in this study, toxicity tests were conducted on individual chemicals, known to be active in this assay. The chemicals are commonly found in water samples and have different modes of action including antibiotics, herbicides, fungicides, antidepressants, metals, among others. In total, 30 compounds were tested, and the best-fitted regression models (Brain and Cousens, Weibull, Log-logistic) were used to estimate effect concentrations and analyse response patterns. Biphasic concentration responses were only present for a few chemicals, and only in the fluorescence assays, leaving the findings of hormesis in absorbance assays on chemical mixtures unexplained. We observed that absolute fluorescence was a more sensitive endpoint for biomass than absolute absorbance for most chemicals. However, this is with few exceptions within the groups of herbicides and antibiotics. This means that the EC50's for fluorescence were mostly lower than for absorbance, varying up to 20%. Furthermore, the relative fluorescence (fluorescence/absorbance) decreases with increasing concentrations after 48 hours for most of the compounds, confirming that fluorescence in general is a more sensitive endpoint compared to absorbance.

2.01.P-Tu032 Reviewing Validity Criteria for *Skeletonema* Sp. Growth Inhibition Studies

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The unicellular marine diatom *Skeletonema* sp. is routinely used to assess growth inhibition often following the ISO 10253 test method. Such data has been compared against the OECD Test Guideline (TG) 201 control validity criteria, which has resulted in inconsistent interpretation regarding whether the data are reliable or not – sometimes in a regulatory context. OECD TG201 is specific to freshwater alga and cyanobacteria - *Skeletonema* sp. is not included as a recommended species. One validity criterion in OECD TG201 (mean coefficient of variation for section-by-section specific growth rates) which is not included in the ISO 10253 standard, is not always achievable for *Skeletonema* sp. The project includes two steps: Task 1: Review of *Skeletonema* sp. control data and culturing techniques to understand the variability in section-by-section specific growth rates and test conditions; and Task 2: Control trials and reference toxicant tests to investigate optimum test design and relevant validity criteria. This project is funded by the Environment Agency, England and undertaken by WCA and Scymaris. Based on the data gathered and generated to date during this project, the mean coefficient of variation for section-by-section specific growth rates in control cultures is found to often exceed the threshold of 35% from OECD TG201, although it clearly can be achieved under certain conditions. This data will help us understand and advise on the best culturing and test conditions for this species, as well as proposing relevant validity criteria for this test. We hope that such an assessment will lead to increased consistency in approaches across different

laboratories and will assist the regulatory review of data submissions. In turn, we hope this will aid evaluation of reliable algal data meaning reducing repeat testing. In addition, the data may be relevant to future test method or guidance document updates which include *Skeletonema* sp. The project completion date is February 2022 and a full review will be available for the SETAC conference in May 2022.

2.01.P-Tu033 Antibiotics in Aquatic Environment: Lemna minor Response to Tetracycline and Recovery After Exposure
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The potential environmental risks of antibiotics are of increasing concern. Antibiotics are recognized as an emerging class of environmental contaminants because of their persistence in the environment and wide use for human and animal applications. The intensive use of antibiotics and incomplete removal from wastewater in wastewater treatment plants leads to their continuous release into aquatic environment. *Lemna minor* were exposed to tetracycline (TC, 1-10 mM) and their physiological status was analysed using the following criteria: rate of plant growth, free radical accumulation, antioxidant enzyme activity, chlorophyll content, HSP70 protein content, cell membrane permeability and mitochondrial activity. The potential of *L. minor* to recover following a 7-day exposure to TC was investigated. Mitochondrial activity was the most sensitive of the studied physiological and biochemical endpoints. The study showed that duckweed can considerably recover from the antibiotic-caused damage, within a week after cessation of the stress. After transferring the plants to a tetracycline-free medium, all plant parameters improved significantly except for mitochondrial activity in plants grown on a medium containing the highest dose of tetracycline.

2.01.P-Tu034 Ecotoxicological Assessment of Rare Earth Elements on the Submerged Macrophyte Myriophyllum spicatum

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In the last decades, the increasing use of Rare Earth Elements (REE) in cutting-edge (smartphone, computer...) and green technologies (wind turbine, solar panel...) led to the release of anthropogenic REE into the environment, and especially in aquatic systems. However, still little is known about the potential effects of these emerging contaminants in the aquatic ecosystems, and more particularly on aquatic plants other than algae. Therefore, we investigated for the first time REE effects towards the submerged macrophyte *Myriophyllum spicatum*. The biological effects of neodymium (Nd), gadolinium (Gd) and ytterbium (Yb) were assessed individually and in ternary mixture in a normalised test system (OECD TG 238) and in mesocosms by measuring plant growth, photosynthetic activity, pigment content and elementary stoichiometry. In both test systems, REE induced hormetic effects on *M. spicatum*, by stimulating growth at lower concentrations and inhibiting at higher concentrations. By modifying test medium composition in the OECD TG, we could show that the type of phosphorous used (inorganic or organic) drastically influenced REE toxicity, suggesting a link with REE speciation and bioavailability. Risk assessment should take into account the type of phosphorous used when assessing REE or other metal toxicity towards plants. In mesocosms, an environmental concentration of REE mixture induced harmful effects by significantly inhibiting *M. spicatum* growth after 4 weeks of exposure. Further biochemical analyses for a better understanding of REE toxicity mechanisms towards *M. spicatum* are ongoing.

2.01.P-Tu035 Long-Term Consequences of Exposure to Nuclear Accident Relevant Radionuclides in Lemna minor From (Epi)Genetic to Community Level Effects

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It is the purpose of this PhD, which is part of the Marie Curie ITN CHRONIC project [1], to study the long-term effects (over multiple generations) at different levels of biological complexity (from molecular to population and community level) in a duckweed species (*Lemna minor*) exposed to environmental relevant concentrations of different genotoxic radionuclides linked to possible nuclear accidental scenarios (¹³⁷Cs, ⁹⁰Sr). The organism *Lemna minor* was chosen for this project as it is a fast vegetative-growing macrophyte, the only freshwater plant for regulatory toxicity testing for chemicals, and a model organisms within ecotoxicology. Moreover, environmental risk assessment approaches used today mainly focus on short-term acute exposures to environmentally relevant radionuclides, however, in field setting organisms are typically (i) in a multiple stressor context, (ii) in communities in which they interact and compete with other species (iii) and are chronically exposed within or even over generations. Therefore, as these approaches do not appropriately cope with complicated exposures at varying degrees of biological complexity, this project aims at contributing to promote a change in the ERA methodologies and practices required to address existing and future contamination concerns. Thus, by employing molecular biology (gene expression analysis) and epigenetic (chromatin compaction and DNA methylation analysis) techniques, and non-conventional endpoint testing, this project aims at expanding current scientific knowledge on *Lemna*'s stress biology and providing new insights and mechanistic understanding of *Lemna minor*'s molecular physiology with particular regards to epigenetic responses towards ionizing radiation. To this end, *Lemna minor* plants were chronically exposed to ⁹⁰Sr for a period of six weeks and samples were taken on a weekly basis to measure parameters such as growth, ⁹⁰Sr uptake and molecular, biochemical and epigenetic endpoints. The experiments and analysis are currently ongoing and the data will be presented and discussed in this poster with particular regards to the relevance for the ecotoxicology field. [1] H2020-MSCA CHRONIC Innovative Training Network

2.01.P-Tu036 Differences of Semi-Static Systems on Growth and Sensitivity of *Myriophyllum Spicatum* Compared to Static Test in Accordance to the OECD 239

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The ring-test on the rooted aquatic plant *M. spicatum* in a water/sediment system was conducted under static conditions. However, guideline OECD 239 also mentions to use systems with several water changes in case of degradation of test item. The basic idea behind is to prove a constant exposure for risk assessment. But an influence on the test system was not clarified and the system is more artificial, since natural degradation was not taken into account. This might under or overestimate effects of degrading test items. Further, water changes influence the pH and might increase the amount of nutrients in the water or wash out nutrients from the sediment. This can lead to a higher or lower sensitivity to chemicals. Stabilisation of test items can also be reached by buffering the test system for pH sensitive test items. We present data with buffered and non-buffered test systems and compare growth of control plants from semi-static and static designs.

2.01.P-Tu037 Comparison of Static, Semi-Static and Flow-Through Test Designs With *Myriophyllum spicatum*

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Testing scenarios considered in the OECD guideline 239 for the water-sediment *Myriophyllum spicatum* toxicity test, are static, semi-static and pulsed dose approaches. For the testing of fast degrading substances, it might be necessary to adapt the semi-static approach to a daily renewal of test solutions to keep up the tests concentrations in the medium and to enable the determination of test item concentrations above the detection limit in the aged solutions. However, even with a daily renewal of test solutions, it happens that test item concentrations in the aged test solutions fall below the limit of detection at the end of the renewal period (24h). A solution to overcome this problem, is to use flow-through systems to keep concentrations stable throughout the test. To evaluate whether a test with *Myriophyllum spicatum* under flow-through conditions can be considered a viable option as an addendum to the OECD 239 guideline, a test has been conducted to compare static, semi-static and flow-through test designs. Results will be presented with focus on potential differences between the test systems concerning plant growth and sensitivity.

2.01.P-Tu038 *Glyceria maxima* As an Alternative in Macrophyte Testing

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Glyceria maxima is currently under evaluation to expand the variety of possible test organisms in a water-sediment toxicity test with rooted macrophytes in addition to *Myriophyllum spicatum* (OECD TG 239). A possible testing approach including the obtained control data will be presented.

2.01.P-Tu039 An OECD Test Guideline for *Glyceria maxima* : Results of a Final Ring-Test

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Registration of plant protection products in the EU requires assessment of effects on aquatic plants and, for some products, tests are required with the emergent, monocot species, *Glyceria maxima*. Since 2014, a Workgroup affiliated to the SETAC Plant Interest Group, has developed a test method in a water-sediment system, which has been evaluated via three international ring-tests. The first ring-test was completed by 13 laboratories during 2016-2017 with the herbicide, isoproturon, and the objectives of establishing the test duration and defining suitable assessment parameters. Results from this ring-test showed that a test duration of 14 days was preferable to 21 days and that leaf length was a more reliable assessment parameter than shoot height. The protocol was adapted to take account of these findings and introduce further standardization in terms of plant propagation methods and test system specification. The revised protocol was used in a second ring-test with the herbicide, imazapyr, in 11 laboratories during 2018. The objective of this ring-test was to determine the feasibility of assessing effects on root growth. Results showed that shoot parameters are more reliable than root endpoints and that further work is required to reduce test variability by improving the uniformity of starting plant material. In April 2019, an OECD Test Guideline project was initiated and a further ring-test was completed in 2021. This presentation will summarize data generated to date and discuss progress towards delivery of an OECD Test Guideline.

2.01.P-Tu040 Screening Engineered and Natural Nanomaterials for Their Effects on Wheat and Lettuce Seed Germination and Nanomaterial Responses to Exposure

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Conventional fertilisers are incredibly inefficient, with up to 60% of nitrogen fertiliser lost to the environment on average, as leachate (e.g. nitrate, ammonia, etc.) and volatilized gases (e.g. NO_x, N₂O) (FAO, 2002). Identifying alternative fertilisation strategies, like use of biochar and engineered nanomaterials (NMs), that are able to minimise environmental losses and prevent long-term effects such as soil acidification, eutrophication, and ozone depletion, while maintaining or improving crop yields is of global significance. Seed treatments are a common method to minimise entry of fertilisers into soil and to prime seeds for better germination and initial growth. Seed germination tests are used to determine NM suitability for agricultural application by assessing the doses at which they display phytotoxicity. We explored *in vitro* the impacts of a broad panel of natural and engineered NMs, including zeolites, hydroxyapatites, biochar, and metal-based NMs, on lettuce and wheat seeds. These seeds

differ in size significantly, which alters their exposure to the NM suspensions, and represent dicotyledonous and monocotyledonous crop plants, respectively. By screening a broad range of NMs with different chemistries it is hoped that more effective comparisons can be drawn between germination rates and NM properties and that NMs' effects can be grouped, and in due course predicted computationally. Only root growth was affected by NM treatment in wheat, with no significant differences found between NM treatments and control for rate of emergence. Lettuce seeds showed a very different pattern of response, having their shoot length affected by NM treatment, and with several NMs that promoted wheat root growth inhibiting lettuce shoot growth. The dose-response seed germination experiment is accompanied by analysis of changes in the composition of the seed exudate and on the impacts of the seed exudate on the NMs, in terms of corona formation and physico-chemical transformation of the NMs, which will help to elucidate the mechanism of effect (enhancing water uptake, intensifying sugar metabolism/energy production, inducing antioxidant defences, and/or remodelling membrane lipids). Results from these seed priming experiments will inform future work to explore the NMs interactions with the two crops, their impacts on nutrient cycling, and changes in the NM characteristics in both hydroponic media and agricultural soils.

2.01.P-Tu041 Multi-Endpoint Toxicity Evaluation of Rare Earth Elements in Terrestrial PLANTS

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Rare earth elements (REE) are a group of 15 elements, the lanthanides and Yttrium and Scandium, with similar chemical and physical properties. They are naturally present in the environment, where accumulation can occur following anthropogenic inputs, altering their biogeochemical cycle. These elements were extracted from complex minerals, and until 30 years ago, they were scarcely investigated. REEs are now considered 'critical' or 'strategic' substances because they are crucial to a wide range of modern technologies such as catalytic additives, hybrid vehicles, wind turbines, oil refining, lighting technologies and, in recent years, have caused widespread concerns about their persistence in the environment and bioaccumulation in the biota. Few data in the literature concern the effect (beneficial or toxic ones) induced by REE application in terrestrial vascular plant species and at different physiological stages. Therefore, the aim of this study was to improve the knowledge on ecotoxic responses to different REEs in terrestrial plants on the basis of their bioavailability in soil and water. To evaluate this, a set of experiments with exposure to different REEs were performed on plant organisms. Preliminary data of investigations focused on the exposure of *Lens culinaris Medik.* seedlings and *Allium cepa L.* bulb to cerium chloride (CeCl₃) and neodymium chloride (NdCl₃) at various concentrations. These acute toxicity tests lasted for 72 hours and results showed alteration of the growth rate and the levels of some parameters considered as biomarkers of stress (reactive oxygen species and antioxidant systems). Some aberrations of the mitosis were also observed in the root tissues of both species. All these analyses are being performed through techniques of spectrophotometry, microscopy, and electrophoresis. Preliminary results indicate that the sensitivity of *L. culinaris Medik.* in this bioassay is higher for lower concentrations compared to *A. cepa L.* Also, lower concentrations of REE had a positive effect on the growth rate of lentils. We will further elaborate on this study and present the first results in the poster.

2.01.P-Tu042 Improving the Interpretation of Terrestrial Plant (NTTP) Toxicity Studies by Considering Crop Tolerance

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Plant Protection Product (PPP) risk assessment to non-target terrestrial plants (NTTPs) needs to account for the rich diversity of the plant kingdom by testing a wide range of potentially sensitive species. Especially herbicides carry a high potential to affect NTTPs and therefore require a careful choice of test species. 6-10 species of different taxonomic groups are usually tested to represent the sensitivity distribution of NTTP communities off-field, mainly crop species. In many cases, labelled crops (i. e. crop plants where herbicide use is intended) need to be tolerant to adverse effects to exclude damage to the crop. Therefore, focusing testing on labelled crop species would severely bias the toxicity estimate. Nevertheless, this case sometimes occurs. As an example, we present the case of a herbicide. It primarily inhibits the seedling emergence/establishment of weeds, but not the crop, which must be tolerant. We show that regulatory studies submitted during the current EU evaluation of the active substance focused on labelled crops and thus on species known to be insensitive to this herbicide. We want to share this assessment to make sure that sensitive species are tested in the studies used to evaluate the PPP risk to NTTPs. A way forward will be presented how to check the tolerance of tested NTTP species exposed to a herbicide to support the design and evaluation of such tests for PPP risk assessment.

2.01.P-Tu043 Identifying Suitable Non-Crop Species for the Potential Use in Guideline Plant Protection Product Testing for the Risk Assessment of Non-Target Terrestrial PLANTS

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To assess the risk of plant protection products (PPP) to non-target terrestrial plants (NTTP), official guidelines provide test designs. A homogeneous germination and seedling growth is of high importance to allow for statistically reliable and reproducible results, accordingly the specified validity criteria of the guidelines require germination rates of at least 70% and a rate of 90% for the survival of emerged control plants. Crops are preferred over non-crop species for testing as they typically show the desired properties. In the past it has been discussed, whether non-crop species are more or less sensitive than crop species and whether the non-crop species are adequately covered within the current PPP risk assessment. At the last SETAC conference (2021), we presented a list of non-crop species which have the potential to fulfil the validity criteria and could be used in guideline testing. However, many of those species (e.g. *M. chamomilla*, *S. media*) can be defined as target species considering the efficacy spectra of common PPP. Target species are not suitable for testing the risk of PPP towards non-target plant species. Thus, this work aims

to identify non-crop species from different plant families which (i) are expected to occur adjacent to agricultural fields in Germany (ii) are not target species, and (iii) can fulfill the validity criteria specified in the guideline. Plant communities (e.g. grass meadows) which would be expected in close vicinity of agricultural fields in Germany were identified. Species from those plant communities were excluded when they were found to be within the efficacy spectrum of common PPP. In total 27 species meeting these criteria were available at seed suppliers. Germination rates and growth stage assessments (BBCH) of species from two suppliers were compared. Two different sowing depth (1 cm of soil and a shallow layer of sand) were applied to identify optimum conditions. Preliminary results showed that depending on the supplier and the sowing depth several species can fulfill the validity requirements for guideline testing. Challenges were seen regarding identifying representative non-crop species, availability as well as quality of seeds and the need for specific sowing conditions.

2.01.P-Tu044 Challenges and Regulatory Relevance of Statistically Assessing Visual Injury As Additional Endpoint in EcoToxicological Testing of Non-Target Terrestrial Plants (NTTP)

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The main refuges for Non-Target Terrestrial Plants (NTTPs) in intensively used agricultural areas are field margins representing semi-natural habitats. Ecotoxicological testing to assess the effects of plant protection products on NTTP's under greenhouse conditions is conducted according to standard guidelines (e.g. OECD 208 and OECD 227). In addition to the quantitatively assessed endpoints mortality or shoot dry weight, visual injuries such as chlorosis, necrosis as well as leaf and stem deformation are also assessed. However, as in most studies for ecotoxicological testing, no statistical estimations (e.g. determining ER_x values) are done for visual injuries. The technical report of EFSA (2019) stated that the ER_x for visual injuries should be reported and used for the risk assessment if this endpoint is the lowest of those available/assessed. However, there are several challenges which have to be addressed before a reliable determination and interpretation of ER_x values for visual injuries could be considered. The objective of this poster is to create awareness for the topic and to discuss the challenges of deriving a reliable endpoint based on visual injury as well as potential solutions. In comparison to quantitatively assessed endpoints such as mortality or shoot dry weight, visual injuries are determined based on different qualitative rating systems (e.g. A-E, 1-4, 10-100%) and a subjective estimation of the assessor. The comparability of visual injury between different studies is therefore challenging. A consistent rating system in combination with a clear guidance would need to be developed for the assessment of visual injuries and to transfer already assessed visual injury data in a comparable scale. From a statistical point of view, the evaluation of visual injury data is challenging because they are neither continuous data like shoot dry weight nor quantal data like survival and they do not allow to account for variability of responses between individual plants from the same replicate. Nonetheless, if it were possible to develop procedures for a reliable estimation of ER_x values for visual injury, the regulatory relevance could be evaluated by comparing it with the currently assessed endpoints shoot dry weight, shoot height or mortality. Currently running initiatives addressing this topic will also be presented on this poster.

2.01.P-Tu045 Protectivity Check of the Tier 1 Pesticide Risk Assessment for Aquatic Primary Producers

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In Europe, the aquatic Tier 1 environmental risk assessment (ERA) of plant protection products (PPPs) for herbicides and plant growth regulators requires the performance of chronic studies with at least two different algae species and one aquatic macrophyte species. These data requirements for active substances are laid down in the Commission Regulation (EU) No 283/2013. According to the Aquatic Guidance Document (EFSA PPR, 2013), the ERA should be based on growth rate related values (i.e., E₁₀C₅₀ for primary producers, with an AF=10), which raised the question whether the ERA for primary producers is still protective after moving from the biomass related endpoints (E_b/E_yC₅₀) to growth rate related endpoints. To further investigate this question, several authors compared the regulatory acceptable concentrations (RACs) resulting from the Tier 1 risk assessment with the Tier 3 RACs resulting from micro/mesocosm studies in the last years. The evaluations available so far have come to different conclusions. Several authors found that, in the majority of cases (van Wijngaarden & Arts, 2018, ET&C 37), the risk assessment is protective while at SETAC Rome the German EPA (oral presentation: Duquesne et al. 2018) presented an evaluation with a different result. In order to investigate this inconsistency, the present study investigated the protectiveness of 18 herbicides and 2 fungicides where primary producers are the most sensitive species. The compounds were chosen considering their use for data comparison in the past and, at least in most cases, Tier 1 and Tier 3 data pairs were available. The Tier 1 and Tier 3 endpoints were extracted from publicly available EFSA conclusions and, if needed, from papers and the resulting RACs compared with each other. For the derivation of the Tier 1 RACs from laboratory studies, the most sensitive species were considered in combination with an assessment factor of 10. To derive Tier 3 RACs from micro/mesocosms, the endpoints were used in combination with assessment factors published in EFSA conclusions, where available on the EFSA website. For some cases, where this evaluation resulted in a Tier 1 RAC being higher than the Tier 3 RAC, and thus giving a hint for non-protectiveness of the Tier 1 risk assessment, we had a closer look on the observed effects in the Tier 3 studies. The in-depth evaluation of the underlying data was used to further increase the understanding about potential risks related to current procedures in the environmental risk assessment of primary producers.

2.02 Arthropods at Risk? Current and Future Perspective on Insect Ecotoxicology

2.02.T-01 Development of an Ecotoxicological Testing Method to Assess Side-Effects of Plant Protection Products on Herbivorous Lepidopteran Larvae Under Laboratory Conditions

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In the current environmental risk assessment of Plant Protection Products (PPP), the exposure route via food ingestion by herbivorous arthropods is not considered and therefore, no standard test protocols for non-target arthropods are available. In the EFSA opinion on non-target arthropods, testing of lepidopteran larvae is suggested to cover this methodological gap, as this group of organisms play an important role in the ecosystem and are widely distributed across Europe. The objective of this study was the development of an ecotoxicological testing method to assess the side-effects of PPP to herbivorous lepidopteran larvae. The presented research was divided into two steps: 1) establishment of *Aglais io*, *Aglais urticae* and *Vanessa atalanta* lepidopteran species rearing under controlled laboratory conditions; a 4th species *Pieris brassicae* was bought from a commercial source as eggs and 2) the assessment of acute and chronic endpoints of these four herbivorous lepidopteran species exposed to two PPP with a different mode of action. Lepidopteran adults and larvae of *A. io*, *A. urticae* and *V. atalanta* were reared under laboratory conditions at 25±2°C, 60-70% relative humidity and a photoperiod of 16:8 (L:D) h. During their larvae stage until pupation, all 3 species were provided with at libitum nettle leaves as food source. Adults were provided with sugar solutions. *P. brassicae* eggs were bought from a commercial source and kept in an environmental chamber at 25±2°C, 60-70% relative humidity and a photoperiod of 16:8 (L:D) hours. Larvae of *A. io*, *V. atalanta* and *P. brassicae* within the age range of 24 to 48 h were used for testing. The test design consisted of a control and seven treatment groups, with 15 replicates each. The test substances were sprayed on the surface of nettle or savoy cabbage leaves, depending on the lepidopteran species tested. Test organism larvae were exposed to treated leaves and kept in test units under controlled environmental conditions (25±2°C, 60-70% relative humidity and a photoperiod of 16:8 (L:D) h). Mortality was assessed 24 and 48 hours after the application of the test substance. Afterwards pupation rate and reproductive performance were assessed. Acute and chronic endpoints will be statistically evaluated and the respective ER_x values will be calculated and presented. It can be concluded that a reliable rearing of the herbivorous lepidopteran species *A. io* and *V. atalanta* can be established as well as the assessment of acute and chronic endpoints of these species is feasible. Challenges of testing herbivorous lepidopteran larvae and the relevance for the risk assessment will be discussed.

2.02.T-02 Evaluation of the Risks of Hydroxymethylfurfural (HMF) for Honey Bees: Results of a Guts-Based Modelling Approach to Assess the Potential for Time-Reinforced Toxicity

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Hydroxymethylfurfural (HMF) is a degradation product of particular sugars and can be present in bee feed. HMF is of low acute toxicity in bees but causes increased mortality upon chronic exposure. Within the frame of an EFSA risk assessment of HMF in bee feed the toxicity of HMF in bees was investigated. Two studies, Gregorc et al., (2020) and Jachimowicz and El Sherbiny (1975) have investigated chronic effects of HMF on the survival of honeybees. Experimental analyses indicated that HMF has potentially time reinforced toxicity (TRT) characteristics. However, the experimental data on HMF in bees might not adequately cover the full life span of summer and winter bees, i.e., the impact related to bee health may be underestimated if the environmentally relevant length of exposure exceeds the duration of laboratory tests. A novel approach was developed, that allows to assess TRT characteristics based on toxicokinetic-toxicodynamic modelling of survival using the GUTS framework. A modified GUTS-RED-SD model was used where everything was standard ([4,5]), except the model for the control mortality, where we used the cumulative distribution function of the log-normal distribution as 2-parameter background mortality model. The parameters of the probability distribution function, p_1 and p_2 , were fitted to the decline in survival over time of the tested bees in the control by minimization of the negative log-likelihood value (see [5]). The remaining 3 parameters of the GUTS-SD-RED model were fitted to the survival over time by minimization of the negative log-likelihood function value. For each data set, the calibrated GUTS was used to calculate survival for a series of 36 exposure levels and exposure durations between 2 and 180 days. Background mortality was not considered for these extrapolations, but alone the toxic effect. The slope of linear regressions of the logarithm of extracted LD50 values for extrapolated exposure duration, and the duration of (simulated) exposure was estimated to a value of -1.89 based on data from Gregorc et al., (2020). The corresponding slope value based on Jachimowicz and El Sherbiny (1975) is -1.96. The TRT characteristic is shown for HMF for both datasets with slope factors s very close to -2; according to the TRT theory, HMF is thus an almost "ideally accumulative toxicant", independent of the specific dataset, and the sensitivity of the test. The developed methodology provides a methodology to analyse the TRT property from data from prolonged ecotoxicological testing by using a modelling approach based on the GUTS modelling framework.

2.02.T-03 Predicting solitary bee pollen consumption and its relevance for pollinator risk assessment

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In recent years there is growing concern that some solitary bee populations are in decline, potentially compromising pollination security in agricultural and non-agricultural landscapes. Among the numerous causes associated with this trend bee exposure to plant protection products in agricultural landscapes has been discussed. Bees can be exposed to plant protection products directly resulting from overspray and/or to residues in pollen and nectar. In the case of solitary bee larvae, the main exposure route is likely pollen and the amount consumed depends on the size of the bee larvae and the pollen composition and (e.g. pollen protein concentration). So far exposure estimates for wild bee larvae for risk assessment purposes have been based on a limited information making their robustness uncertain. In an attempt to tackle this question we combine published information on solitary bee ecology (plant preference), plant pollen quality (pollen protein concentration), bee larvae weight and pollen consumption to

build a inter species correlation model to estimate the protein needs of solitary bee larvae. By combining this model with known pollen protein content, we predicted the expected pollen needs of a developing red mason bee (*Osmia bicornis*) a regulatory relevant pollen generalist (polylactic). In summer 2020 we tested the model predictions in a field experiment in central Germany. These results support the validity of the model framework. We discuss our findings in the context of current EU risk assessment framework and highlight the potential of using scaling models to support pollinator risk assessment.

2.02.T-04 Concept for a Geospatial Landscape Model to Estimate the Site Specific Forage Supply for Pollinators

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Key pollinators, such as honeybees, face multiple stresses (food shortage, pesticides, diseases). To address these problems, the landscapes complexity needs to be regarded as a whole. Vegetation and especially (semi-natural) elements such as grassy field margins and hedges play a key role. They need to be considered to understand temporal and spatial resource availability and to assess potential resilience and/or recovery of pollinator populations. Process-based modelling techniques are recommended to cope with the high complexity of the landscape. We here present a spatially explicit modelling approach that estimates the forage availability for pollinators in an existing agricultural landscape throughout the year. In addition to the amount of forage provided by agricultural crops, the supply by semi-natural vegetation in off-crop areas is estimated based on existing GIS (geoinformation system) data. To this end, we set up a data base with GIS data for the given landscape (e.g. cadastral maps, landscape elements), phytosociological data on vegetation units, and plant trait data (e.g. forage supply, flowering time), estimated the present vegetation units based on the GIS data, estimated the species cover for the vegetation units based on the phytosociological literature data, calculated the nectar and pollen supply of the given vegetation unit throughout the year and created a forage supply map of the considered landscape. We used this map as input data for the simulation model BEEHAVE, which simulates the development of a honeybee colony and its foraging of nectar and pollen in different landscapes. The developed modelling approach can be added as a modul to the dynamic GraS-Model to allow for a process-based plant community modelling including the forage supply for pollinators. The presented research is part of the ongoing project VIBee (establishing digital indicators of bee vitality in the agricultural landscape, <https://vibee-project.net/>). Further research will include the analyses of the following collected data: counting of a colony's flight activity using electronic counters (BeeCheck), the weight of the beehives (5 minutes resolution), weekly analyses of the collected pollen, field data of surrounding vegetation including flowering plants over the vegetation season. The here presented modelling approach can be adjusted for other pollinators and other landscapes.

2.02.T-05 Variability in Chemical Sensitivity of Arthropod Communities Across Europe

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Current chemical risk assessment with respect to chemical effects on non-target organisms largely follows a one-size-fits-all approach, focusing on sensitive single species tests or multispecies test systems. However, real world species assemblages vary in their composition over space and time and so may their sensitivity towards a chemical. Depending on the variability in sensitivity, the use of a single effect threshold can result in over- and underprotection. Hence, knowledge on this variability is pivotal for an efficient risk assessment. We examined the sensitivity of selected aquatic (freshwater invertebrates) and terrestrial (spider) arthropod communities towards four chemicals with different modes of action (herbicide, insecticide, metal, pharmaceutical) across Europe. We compiled related monitoring data covering major macroecological gradients in Europe and used biogeographical and habitat classification systems to derive typical assemblages for freshwater invertebrates and spiders. Subsequently, we estimated the chemical sensitivity based on statistical models and derived sensitivity distributions for the different assemblages. Our results show that assemblage sensitivity can vary considerably, in particular in freshwater invertebrates. We discuss the implications of our results for arthropod conservation and chemical risk assessment.

2.02 Arthropods at Risk? Current and Future Perspective on Insect Ecotoxicology (Virtual Only)

2.02.V-01 Estimating the Range Shift of Beetle Occurrence Using Species Distribution Modeling - an Application in the Climate-Change Perspective

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With a changing climate, many species, including agricultural pests, are likely to shift their geographical ranges. This has important implications for pest management and crop yields. How could this shift look like for major pests? To get an idea for a specific example, we analyze the occurrence data of the spotted cucumber beetle (SCB), a significant pest to cucurbit crops in the US. The purpose is to (i) explore the presence of suitable habitat in the USA and (ii) project the habitat suitability to the future, considering different climate change scenarios. The work is performed using a flexible and open platform for modeling species niches and distributions. To build a species distribution model, data on species occurrence (locations where the species was observed) and environmental conditions relevant to the species across the US have to be retrieved and processed. For our analysis, we used the dataset of the SCB occurrences from the Global Biodiversity Information Facility and the environmental data from WorldClim. After processing these datasets, we performed a partition of occurrence data to test the accuracy of the predictive

model. Subsequently, we ran the species distribution model which is based on a machine-learning algorithm that estimates the species response to the environment. Model outputs show the relative abundance or probability of occurrence over space (the USA) and time (projections to 2070). We tested two different scenarios of climate change using the intermediate RCP4.5 scenario and the worst-case RCP 8.5 scenario. Results suggest that there will be areas of estimated range loss of SCB (Southeast US, Southern Texas) and areas of estimated range expansion (Northeast, Northern parts of the Midwest, Eastern regions of CA, and the Pacific North West) in both climate scenarios, suggesting that the species could become a major pest in regions where it is currently absent or occurring at low abundances.

2.02.V-02 Evaluating the Effects of Fungicide and Herbicide Alone and in Binary Mixture on *Apis mellifera* by a Multi-Biomarker Approach

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Apis mellifera plays an important role in agriculture and in the conservation of plant biodiversity. This species is disappearing globally due to parasites infestations and diseases, habitat loss, beekeeper management issues and to the widespread use of pesticides. Honeybees could be exposed not only to single pesticides but also to their mixtures that may cause synergistic or antagonistic effects. Insecticides and their sub-lethal effects have been widely studied in this species, while fungicides, herbicides, and pesticide mixtures, are not equally investigated. The main objective of this work was to investigate the sub-lethal effects in *Apis mellifera* after the exposure to two doses of a commercial fungicides (having as active principles 15.86% bromuconazole + 10.17% tebuconazole), of a commercial herbicide (2,4 D), and to the combination of them, by applying a multi-biomarker approach. A control with acetone was used for each experiment. The specimens were exposed topically to two concentration of the fungicide (200 g/L and 400 g/L), of the herbicide (225 g/L and 450 g/L), and of the binary mixtures of pesticides (200 g/L fungicide + 225 g/L herbicide and 400 g/L fungicide + 450 g/L herbicide). The experiment lasted 4 days. AChE, CaE, GST, ALP, and lysozyme activities, hemocytes differential count and NA assay were evaluated. CaE was inhibited in both fungicide treatment groups. Since AChE did not show a neurotoxic activity, it is possible to hypothesize that fungicide may act at metabolic level. GST showed an induction at the lowest dose respect to control, while at the highest dose the activity decreased; fungicide may activate organisms' biotransformation systems response. Erbicide highest dose showed an increase in GST and a dose-dependent inhibition in ALP. It is possible to hypothesize that herbicide modulates organisms' biotransformation and metabolic responses. CaE showed an inhibition at the highest mixture dose while GST showed an increase at the same dose. Mixture lowest dose showed a slight decrease in ALP activity respect to the control. Mixture highest dose showed an increase in the granulocytes number. The results obtained after the treatments with these commercial pesticides alone and in binary mixtures showed that they can modulate the detoxification and metabolic responses. Moreover, the multi-biomarker approach proved to be a useful tool for the ecotoxicological evaluation of xenobiotic compounds effects in pollinator species.

2.02.V-03 New Method for Evaluation of Damaged Digestive Tract in *Collembola Folsomia candida*

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Folsomia candida is widespread arthropods and commonly used test species in soil ecotoxicology, therefore the test guidelines for *F. candida* have been developed to assess the survival and reproduction. However, there are limited method for evaluation of toxicity of chemicals in individual level even though collembola are becoming an increasingly important subject in soil ecotoxicology area. The purpose of this study was developing a new method into soil ecotoxicological evaluation of chemicals using *F. candida*. This study hypothesized that damaged digestive tract of *F. candida* can be visualized by trypan blue which staining dead cell. To find the optimum staining media, staining duration and dye concentration, necessity for agar, sugar, various staining duration (5 to 120 minutes), and dye concentrations (0.1% and 1%) were investigated and compared. Subsequently optimum test conditions using trypan blue were applied to metal exposed *F. candida* to confirm applicability of this new method. As results, we found that a new method supported to evaluate and visualize the digestive tract damages in *F. candida* after metal exposure. We expect this new method for evaluation of damaged digestive tract using trypan blue can contribute to expand the test endpoints for collembola *F. candida*. *Acknowledgement-This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2020RIA2B5B02001734, 2021R1C1C2012628)*

2.02.V-04 Sensitivity of Bee Species to Pesticides: A TK/TD Perspective Using the GUTS Modelling Framework

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Although empirical data on non-apis species is being generated lately, risk assessment for bees is still for a large extent based on data for honey bees. This raises the question whether or not data for honey bees are a good proxy for other bee species. This question is not new and has resulted in several publications where the sensitivity of bee species is compared. In all publications, comparisons are based on 48 *LD*₅₀ values from acute contact test results. In such a test, a drop of fluid containing the compound of interest is placed in the neck of the bee and subsequently effects on survival of the test subjects are observed, the test result of which is summarised in the 48 hr *LD*₅₀. However, previous research showed that during this observation period, the exposure concentration declines over time. This in itself raises questions on the validity of the *LD*₅₀ value and in addition, the decline over time is likely to be different for different species, depending on the characteristics of the bee. Therefore an assessment of bee sensitivity based on 48 hr *LD*₅₀s is problematic. These issues can be taken into account by the use of a Toxicokinetic/Toxicodynamic (TK/TD) model that can handle time-varying exposure concentrations. The endpoint then becomes a time- and test-

independent measure for the toxicity; the effect threshold. This approach was tested and validated for honey bees in the so-called BeeGUTS framework, but so far the approach has not been applied to species other than honey bees. This framework was now calibrated and validated for different species of bees (*Osmia*, *Bombus*, *Megachile*) and a variety of different pesticides allowing for the comparison of bee sensitivity and the evaluation on whether or not the honey bee is a good proxy for bee sensitivity in environmental risk assessment.

2.02.V-05 Temperature Sensitive Effects of the Neonicotinoid Clothianidin on Bumblebee (*Bombus terrestris*) Foraging Behaviour

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Currently, 46% of European bumblebee species have declining populations. Pesticide usage has been pointed out as one of the drivers behind the decline of wild bee populations. Globally, neonicotinoids are one of the most commonly used insecticides. Studies have shown that exposure to neonicotinoids at sublethal concentrations can alter foraging behaviour of pollinators. Bumblebees are social insects with a complex colony structure and division of labour. Any disturbance of these intricate activities might negatively affect bumblebee colony survival and reproduction. As there is still little knowledge about how interactions between anthropogenic stressors such as neonicotinoids and natural drivers such as ambient temperature affects bumblebees, the aim of this study was to assess if foraging bouts were affected by clothianidin, and if this effect was modulated by the ambient weather conditions (i.e., temperature and precipitation). Using a custom-made bumblebee colony monitoring system, we examined how the number and duration of foraging bouts of bumblebees on an individual level was affected by exposure to low (6.5 µg/L) and high (10.6 µg/L) sub-lethal concentrations of the neonicotinoid clothianidin via nectar. We also examined possible interaction between clothianidin exposure and abiotic factors (temperature and precipitation), and its impact on foraging bout number and duration. Exposure to sublethal concentrations of clothianidin increased foraging bout duration in bumblebees. Furthermore, the foraging bout duration decreased with increasing temperature at both exposure concentrations, whereas the unexposed control group was not affected by temperature. Neither number of foraging bouts nor the daily rhythm of foraging bout duration was affected by clothianidin exposure or temperature. The foraging bout duration decreased with increasing precipitation in both exposed and non-exposed groups. However, we did not find any interaction between precipitation and exposure, suggesting that precipitation does not affect toxicity. Our study shows the importance of semi-natural experiments and accounting for ambient factors when assessing the risk that pesticide exposure may present to pollinators. We conclude that the effect of clothianidin exposure on bumblebee foraging behaviour is temperature sensitive and that local climatic conditions and future climate change scenarios should be considered in risk assessments of clothianidin and other insecticides.

2.02 Arthropods at Risk? Current and Future Perspective on Insect Ecotoxicology (Poster)

2.02.P-Mo061 A Chronic Toxicity Test With the Mayfly *Cloeon dipterum*, Results of a Ringtest

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In recent years, the interest of testing non-standard species for environmental risk assessment of pesticides has increased. Particularly, mayflies gained interest due to their relevance in aquatic ecosystems and sensitive response to chronic exposure to certain pesticides. In this context, we developed and refined a chronic test system for the mayfly *Cloeon dipterum*. To evaluate the validity of the test procedure among laboratories, a ring test comprising six participants was carried out. The participating laboratories performed the test according to the same protocol with local larval wild catches of *C. dipterum*. Larvae were exposed to at least five concentrations of Imidacloprid in a semi-static test design. The larvae were preferably fed with lab grown periphyton on unglazed tiles. Tiles were exchanged at least once a week ensuring provisioning of food ad libitum food. In some labs additional experiments with artificial fish food were performed. The experiments were terminated when almost all larvae had emerged or died. The larval development was monitored two to three times a week by the determination of the developmental stage. All laboratories were able to determine the developmental stages as well as immobility. In four of the five laboratories that carried out experiments with periphyton, the test duration and thus the development rates of the larvae were comparable with control mortalities not exceeding 30% at test termination. Here, mortality was observed predominantly for older larvae and nymphs. Larvae fed with artificial fish food developed slower and showed higher control mortality relative to the provisioning of periphyton as food source. Also, the effect data show a clear dose-response and are comparable between the laboratories. Thus, the results give good indications for further development of the test protocol. Overall, the results are very promising and represent an important step forward in the development of a robust test method for mayflies. **Acknowledgement** - The authors thank Julius Alberti, Margret Arnold, Marie-Claire Boerwinkel, Marie Brown, Mirco Bundschuh, Noel Diepens, Alexander Feckler, Jutta Hager, Laura Koster, Charlotte Krapp, Sophie Oster, Sebastian Pietz, Joe Reiser for their assistance in performing the tests.

2.02.P-Mo062 Behavioural Ecotoxicology: A Novel High-Throughput Approach to Quantify Sublethal Effects

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There is an increasing demand for the implementation of sublethal effects in chemical risk assessment over the last years, not at last because sublethal endpoints are considered more sensitive than traditional endpoints in acute or chronic toxicity tests. However, the assessment of sublethal endpoints faces the problem of standardisation. To address this problem, we apply an approach that uses high-throughput data collection and allows us to quantify behavioural changes in real-time time with an optical behaviour monitoring tool. Our promising approach has the potential to contribute significantly to the understanding, standardisation and incorporation of sublethal behavioural effects into risk assessment. In our pilot study, we used *Gammarus* spp. due to their important ecological role in European freshwater systems and quantified their behavioural patterns under chemical exposure. Behavioural measures included locomotion activity, mating or foraging behaviour, which are elemental to the survival of the individual or the persistence of the population. Identifying abnormalities in these essential behavioural traits is a major challenge, especially in the context of multiple interacting stressors such as climate change, anthropogenic chemical pollution and the competition with invasive species. In our study, we have simulated a pollution wave and recorded the swimming activity of the individuals for 90 min while they were exposed to different pharmaceuticals and pesticides in sublethal concentrations. Additionally, an acetylcholine esterase inhibition assay was conducted to differentiate between a stress reaction and neurotoxic activities of the compounds. Our experiments revealed that even in low concentrations of ibuprofen (1ng/L), methiocarb (1.88 µg/L) and dichlorvos (1.56 µg/L), the test substances affected the behaviour of the gammarids. Our approach shows the enormous potential of behavioural ecotoxicology and the need to develop high-throughput and standardized assessment tools. With the right tools it is feasible to create a comprehensive, inter-disciplinary, multiscale approach to systematically identify behavioural endpoints and implement them in the risk assessment of substances. In this presentation, we give an overview of the method using the two pesticides dichlorvos and methiocarb as examples, the insights and our plans for future research on behavioural ecotoxicology of amphipods in the evolving Anthropocene.

2.02.P-Mo063 Ecological Significance of a Pesticide-Induced Geotaxis Reversal in Mosquito Larvae (Culicidae)

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Fourth instar *Culiseta longiareolata* larvae were exposed to imidacloprid (0, 1 and 2 mg/L) and filmed for 10 minutes. The time spent in negative geotaxis was the cumulative time the larvae spent breathing at the water surface and moving upward within the water column. The time spent in positive geotaxis was the cumulative time the larvae spent moving downward below the water surface or resting at the bottom of the water column. We also assessed the larvicidal effects of imidacloprid after exposure to 0, 0.5, 1, and 2 mg/L for 24 hours. In the control, the larvae spent significantly more time in negative geotaxis, assuming the breathing stance 75% of the time ($p < 0, 01$). In the presence of imidacloprid, a reversal of geotactic behaviour occurred, with a significant decrease in negative geotaxis in all treatments ($p < 0,05$) and a significant increase in positive geotaxis in the 1 mg/L treatment ($p < 0,05$). Fitting the geotaxis data onto polynomial functions, helped estimating the duration of exposure (i.e., 300 s) and imidacloprid concentration (i.e., 0,523 mg/L) beyond which the behaviour of the larvae switched from negative to positive geotaxis. The 0,523 mg/L (concentration at the behavioural tipping point) was similar to the 24h LC₃₁ of 0,519 mg/L. Considering the mode of action of imidacloprid, in nature such a reversal in behaviour could cause greater mortality than 31%. More research is needed to establish whether these preliminary findings and testing framework could find applications in mosquito control programmes.

2.02.P-Mo064 Common Practices to Promote Beneficial Insects and Biodiversity in Vegetables Producers in Almeria

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The cultivated area of Almeria consists of ca 33,000 hectares dedicated to the cultivation of pepper, tomatoes, zucchini, cucumbers, watermelons, melons and aubergines, under greenhouses. Greenhouses are typically considered artificial environments where biodiversity is highly reduced, and where biocontrol is usually provided by the grower. However, measures can be implemented to bring biodiversity back into these cultivated areas, which are supported by national and European programs. The regional research centers IFAPA de La Mojonera (Junta de Andalucía), Las Palmerillas (Cajamar) and farming associations COEXPHAL (fruits and vegetables growers association of Almería) and APROA (fruits and vegetables growers association of Andalucía) foster the implantation of these new techniques. One of these techniques consisted in the implementation of ca 200 km of new green perimeter around greenhouses, making a total of 5-7 % of the greenhouses holding exterior vegetation. These hedges act as a refuge and source of feed (nectar, pollen and other preys) for auxiliary fauna, as well as a barrier against pests and finally improving the agricultural landscape. To ensure diversity and sequential flowering seasons, at least five different species are typically used chosen from lavender, rosemary, mastic, sea parsley, thyme, matagallo, broom, esparto, myrtle, daisies or wild olive. In some cases, these bushes will become orchards, where small birds and reptiles will establish. Inside the greenhouses, appropriate plants acting as reservoirs or multiplication spaces for auxiliary insects are installed, such as fennel, sage, corn, yarrow, calendula, marigold, sunflower or lobularia. The objective is to get a non-stop flowering vegetation that can be used as feed or as hosts for different auxiliary insects. It is estimated a 15 % of the greenhouses already use these reservoir plants inside. Additional tools have been developed as the mobile app, PlantEN, designed to identify the most convenient plants to be used as natural reservoirs. Additional applications such as IPM Pro are available that guide farmers on the crop protection products that are compatible with beneficial insects and biological control. This poster will illustrate in situ implementations of the technique and results in terms of biodiversity gains.

2.02.P-Mo065 Feeding Effects of a Systemic Insecticide on Ant Workers and Larvae

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Neonicotinoids are highly effective insecticides. Due to their high solubility in water, they are absorbed by the treated crops and move within the plants vascular system which can result in their presence in all parts of the plant. Neonicotinoids are known for high toxicity to bees which can result in sublethal effects also at relatively low exposure levels. Due to their close taxonomic relatedness to bees, presence in or close to agricultural areas and similar social organization, ants (Formicidae, Order Hymenoptera) might also be sensitive. Many European ant species are omnivore and general scavenger. Thus, their exposure routes might be different from bees as exposure via food chain is more relevant for ants. Using pest insects, which were killed by an insecticide, as food source could lead to the uptake and distribution of the insecticide in the whole colony because many ants use trophallaxis, a method to share stomodeal fluids with nestmates. Hence, brood and queens are possibly affected. They depend on more resources than adult workers due to growth, development and (for queens) reproduction. We will present the first results of experiments using a systemic insecticide, imidacloprid, to test the effects of exposure via the diet on workers and larvae of *Camponotus maculatus* (carpenter ants) as surrogate test species. *C. maculatus* is an omnivore species and belongs to the subfamily Formicinae, which is the dominant ant subfamily in Europe (e.g., *Formica*, *Lasius*, *Camponotus*). Colonies can be easily maintained in the laboratory over many years and thus, test organisms are available all over the year. The test item will be applied to the workers solved in sugar solution. In a first test, acute effects on workers will be tested. In a second study, we will investigate the transfer of the test item to the larvae via trophallaxis. Therefore, behaviour and survival of worker as well as growth, development and survival of larvae will be assessed.

2.02.P-Mo066 How Can Ants Be Exposed to Plant Protection Products and Transgenic Plant Toxins?

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Ants occupy different ecological niches and provide important ecosystem services as waste decomposition, soil formation, and feed provision for other animals. They live in many different types of habitats, including agricultural areas. However, up to now ants are not explicitly assessed in the environmental risk assessment for plant protection products or transgenic plants. Because it is not feasible to test all taxonomic groups on a routine basis, the risk assessment is based on surrogate species and it is assumed that by the use of safety factors, also not tested taxa are sufficiently protected in the field. Honey bees, bumble bees and wild bees, can be principally considered to be suitable surrogate test species for ants in ecotoxicological tests since they belong to the same insect order of Hymenoptera and are (partly) also social insects. Nevertheless, the exposure routes of ants and bees might be different. For example, ants might collect pest insects killed by plant protection products or toxins produced by transgenic plants and use these to feed larvae or queens. Thus, the aim of this project is to explore the relevant exposure routes for ants in agricultural areas in the EU, to identify suitable test species and to conduct tests of different complexity as case studies with plant protection products, toxins produced by plants or transgenic plant material itself. We will discuss which ant species are expected to be present in agricultural areas in the EU and thus, are potentially exposed and which exposure routes to plant protection products and toxins of transgenic plants can be relevant for ant species with different feeding strategies.

2.02.P-Mo067 Reproduction of Chrysoperla and Coccinella - Only Qualitative or Ready to Use for the Risk Assessment?

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As summarised in the EFSA Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods (2015), the ladybird beetle species *Coccinella septempunctata* and the lacewing species *Chrysoperla carnea* are foliage dwelling predators already selected as relevant test species in the ESCORT 1 guidance document (1994). In the IOBC approved test guidelines, both species are listed and published by Schmuck et al. (2000) and Vogt et al. (2000). Whereas the statistical evaluation of the endpoints of mortality and reproduction are required for many selected non-target arthropod species (e.g. *Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Aleochara bilineata*), for *C. septempunctata* and *C. carnea* only mortality is the endpoint to be statistically analysed. In contrast, reproduction is defined as a “qualitative” endpoint for these two species without the need of statistical testing, as “for the time being, the reproductive performance is evaluated only qualitatively due to the high species-inherent variability in egg-laying performance” (IOBC 2000). From our point of view, reproduction as sublethal parameter can be highly valuable and would increase the significance of the assessment of possible effects of a plant protection product. It is acknowledged that the limited number of replicates in the reproduction phase in *Coccinella* and *Chrysoperla* tests does not allow a valid testing of statistically significant differences between the treatments and the control. However, the establishment of a rate-response relationship allows the determination of the effect rates with 10 % effect (ER₁₀) and 20 % effect (ER₂₀) which supplement the No-Observed-Effect-Rate (NOER). Based on the data sets of two independent laboratories, the significance of the rate-response approach for the potential risk of plant protection products on reproduction of the foliage dwelling predators *C. septempunctata* and *C. carnea* is evaluated.

2.02.P-Mo068 Fitness and Life History: Incorporating Sublethal Effects on Invertebrates Into Risk Assessment of GMO Toxins

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The release of genetically modified organisms (GMOs) is regulated in the EU (Directive (EC) 2018/350) and potential risks to the environment must be assessed depending on the type of genetic modification and before a release of GMO into the environment. Many transgenic crops express proteins with biocidal properties, e.g. to protect the crops against insects, other pests or pathogens. The main route of exposure of non-target organisms (NTOs) to such toxins is uptake via the diet, either directly by feeding on living or dead plant material or indirectly by biomagnification within the food chain. Ecotoxicity tests are central to identify hazards for NTOs and most tests focus on lethality. At lower doses, stressors may also cause sublethal effects which can be biologically meaningful and negatively affect the sustainability of populations. From this perspective sublethal effects should, and in fact are foreseen to be included in the overall risk assessment. However, the number of test methods to assess sublethal effects, especially resulting from uptake with the diet, is limited. With the current project we focus on non-target invertebrates and want to screen how methods for the assessment of sublethal effects used for other stressors such as plant protection products, may be applied or modified to the assessment of GMO for the oral exposure route. The second aim is to evaluate how life-history theory, including the importance of constraints and trade-offs, may be informative for the assessment of sublethal effects. The results of the literature review will be used to develop a first proposal how sublethal effects on invertebrates could be better included in the risk assessment of transgenic crops.

2.02.P-Mo069 Bee-Longing Together - Using BEEHAVEecotox for a Better Understanding of (semi-)field-studies and Testing of Mitigation Strategies

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Insect pollination is an important ecosystem service and pollinators play an essential role in providing important pollination services to most wild plant species and cultivated crops. Thus, pollinators and as such honeybees, are a crucial part of the environmental risk assessment of pesticides in the European Union. In this context, mechanistic modeling offers a powerful tool to predict the exposure and effects on bees in the field. The BEEHAVE_{ecotox} model is the first model to mechanistically link the realistic exposure in the field with subsequent effects on different levels of the bee colony. We use the BEEHAVE_{ecotox} model which is implemented as an extension of the honeybee colony model BEEHAVE in NetLogo. BEEHAVE_{ecotox} consists of 4 modules: the exposure module, the water foraging module, the in-hive fate module, and the effect module. The model was setup to represent the conditions of (semi-)field studies in terms of number of adult bees, brood, honey and pollen stores, forage availability, and weather conditions. For this case study an insecticide application was simulated and the effects on the colony strength were assessed based on different mitigation strategies and (semi-)field conditions. The results show how BEEHAVE_{ecotox} can be used to help better understand effects and dynamics observed in (semi-)field studies. The model can be used to assess the cascading effects on different cohorts, such as foragers, on the longterm dynamics of the honeybee colony. The case study further investigates how BEEHAVE_{ecotox} can be used to test different mitigation strategies. Strategies can include application timing, such as application during day or night to avoid contact exposure. Furthermore, the BBCH stadium of a plant can be taken into account, e.g., via pre- or post-flower applications. Bees play a crucial role in the pollination of our agricultural products and thus it is important to be able to predict the exposure and effects on bee colonies in the field. The BEEHAVE_{ecotox} model is a suitable and validated tool to predict the effects of plant protection products on bees. It is the first model to mechanistically link exposure and effects and predict PPP exposure both outside the hive to foragers and within the hive from several routes of exposure. In this study we will show how such a model can be used to better understand (semi-)field studies and to test different mitigation strategies in the context of environmental risk assessment of honeybees.

2.02.P-Mo070 Predicting the Outcomes of Honey Bee Semi-Field Studies Using BEEHAVEecotox

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Factors affecting honey bee health are manifold (including diseases, parasites, pesticides, environmental and socio-economic factors). The deriving complexity for the environmental risk assessment (ERA) of plant protection products (PPPs) for bees is currently assessed at the higher tier using semi-field and field experiments often conducted with honey bee colonies. The inability to conduct such studies for all plant protection products, their different use patterns, and at different climatic zones makes coherent availability of data and their interpretation and the use for higher-tier risk assessment challenging. There is a consensus amongst different stakeholders that mechanistic modelling can strengthen the ERA for pollinators. Currently, the BEEHAVE model is a freely available and potentially suitable candidate. Its potential suitability has recently been increased by the addition of a mechanistic ecotoxicological module. We present the results of a BEEHAVE_{ecotox} evaluation study conducted by comparing model simulations with observations made in three semi-field studies with tunnel setup. These semi-field studies were carried out to assess the impact of spirotetramat applied on different crops on bee colonies. The general tendency of the model predictions across the studies was to overestimate the impact of the PPP on the total colony strength. This observation is in line with the desired conservatism enforced into the conceptual development of the ecotoxicological module for BEEHAVE. Overall, the observed discrepancy between model predictions and experimental observations can be explained by the lack of empirical data on the food availability for model parameterisation, particularly in the post-exposure phase (landscape characterisation). The lack of information on food availability affects the model prediction for pollen and nectar resources in the hive, which causes the discrepancy in the abundance of bees. Additionally, standard semi-field studies lack precise information on the colony structure, which might cause additional inaccuracy in the model prediction. The addition of a mechanistic toxicological module that accounts for toxic impacts on adult and larva survival amplifies the potential use of BEEHAVE_{ecotox} for higher-tier ERA. The

parameterisation of the combined model can be lengthy and challenging. However, parameterisation of the ecotoxicological module is straightforward as standardised experimental data are available and no other information is required. Parameterisation of the combined BEEHAVE_{ecotox} model is only required for model evaluation studies. Once there is abundant evidence that the model serves the purpose for ERA, standardised simulation scenarios can be developed. Further, BEEHAVE_{ecotox} parameterisation for ERA purposes will no longer be a challenge.

2.02.P-Mo071 BEEHAVE and Brood Termination Rate - a Modelling Study How Magnitude and Duration of Effects Determine Colony Strength

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The brood termination rate (BTR) investigated in OOMEN and OECD GD 75 studies for pesticide risk assessments is the determinant of honey bee (*Apis mellifera* L.) mortality during development from egg to adult and thus influences colony strength. Colony strength as the number of worker bees per colony affects pollination services, yield of hive products and colony viability. In this context, a honey bee colony is regarded as viable and strong enough for successful overwintering and subsequent development to a vital colony in the following year, if at least 5000 worker bees are recorded prior to hibernation according to the respective EFSA Bee Guidance Document of 2013. The EFSA bee guidance gives levels of forager mortality due to pesticide exposure at which colony strength is assumed to fall below this threshold. Impacts of pesticides on honey bee brood are commonly investigated under semi-field, worst-case exposure conditions according to OECD GD 75 with the BTR as one key endpoint. Until now it remains unclear how magnitude and duration of effects on the BTR affect the strength of honey bee colonies before overwintering and thereby the hibernation ability and viability in the following season. Using the honey bee colony model BEEHAVE, we simulated how BTRs of different magnitude and duration affected colony strength prior to overwintering. Our results show how different BTR values influence the colony size, aiding the interpretation of experimentally observed BTRs in terms of consequences for colony strength and viability.

2.02.P-Mo072 BEEHAVE_{ecotox} - a Mechanistic Effect Model for Honeybees

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Insect pollination is an essential ecosystem service. Thus, pollinators and as such honeybees, are a crucial part of the environmental risk assessment of pesticides in the EU. Here, we introduce BEEHAVE_{ecotox}; an ecotoxicological model that mechanistically links exposure of bees in the field with the hazard profile for individual honeybees, leading to emerging colony effect. It is an addition to the extensively tested and widely used BEEHAVE colony model. The mechanistic link allows the translation of results from standard laboratory studies to processes and parameters relevant for simulating bee colony dynamics. The BEEHAVE_{ecotox} model includes 4 submodules: an external exposure module, in-hive fate module, water foraging module, and effect module. The external exposure module incorporates the concentration of PPPs in the bee-relevant matrices such as nectar, pollen, and water. When foragers forage on these matrices, they receive an oral dose of the PPP. Foragers can also be exposed via contact on the day of application. The water foraging module incorporates the water need for dilution of stored honey and cooling of the hive, including potential exposure to PPPs. The in-hive fate module simulates the entry and mixing of the PPP into the hive, through nectar, pollen, or water. The effect module includes the mortality due to exposure for the different cohorts. It uses the slopes and LD50 values of standard acute contact, oral, chronic oral, and larvae studies as inputs. The BEEHAVE_{ecotox} model was validated against two semi-field studies with a tunnel setup with two PPPs with different modes of action (dimethoate and fenoxycarb). The validation showed that the BEEHAVE_{ecotox} model captured the initial effects on colony strength and the subsequent colony dynamics well for both substances. The model predicted the relative magnitude of effect at colony level directly after application, as well as the long-term reduction in colony strength in the post-exposure phase after the tunnel and the lack of recovery of the colony. The BEEHAVE_{ecotox} model is a suitable tool to predict the effects of PPPs on bees. It is the first model to mechanistically predict PPP exposure to foragers and within the hive from several different routes of exposure. For the regulatory risk assessment the model can potentially be used to extrapolate from laboratory to semi-field and field studies, study the effects in different crops and regions, and to test different mitigation strategies.

2.02.P-Mo073 Impact of Plant Protection Products and Nutritional Resources on Bumble Bee Colony Development and Their Microbiome

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Bumble bees are exposed to multiple stressors, including exposure to agrochemicals, low food resource quality and parasites and pathogens. These stressors can affect bee health and vitality. In conventional agriculture application, plant protection products (PPP) play an important role to ensure production quality and quantity. During their foraging flights, bees and other pollinators are exposed to PPPs, either to single products or mixtures of PPPs. The objective of this study is to investigate the interaction between different stressors (different nutritional status and exposure to a mixture of PPPs) and the related impacts on bumble bee colonies and their microbiome under semi-field conditions. The semi-field study was realized with twenty tunnels: ten tunnels planted with the bee attractive sweet lupine (*Lupinus albus*) and ten tunnels planted with lupine and a flowering strip. Additionally, half of the tunnels were sprayed with a tank mixture of the fungicide prochloraz and the insecticide chlorantraniliprole. In every tunnel we placed two small queen-right colonies with the bumble bee *Bombus terrestris*. Various parameters were investigated such as mortality of adults, colony development and the composition of the microbiome in larvae,

adult guts and involucrem. Overall, mortality of adults in the treated variants (lupines and flower strips) was significantly higher compared to the control. The median mortality in the controls three days after application was 2 bees/colony compared to 12 bees/colony in the treated variants. In treated lupine, we found in median 6 dead bees/colony whereas in treated lupine with flower strips we found 21 dead bees/colony. The different mortalities in flower strips and lupine might be explained by a higher flight activity in flower strips. Number of young brood (eggs and larvae) did not differ between variants. However, the number of pupae in lupine was significantly lower compared to variants with flower strips. In combination with PPPs, the number of pupae shows significantly negative interaction effects. Our results show that the mixture of chlorantraniliprole and prochloraz has a negative effect on worker mortality, but not on brood, except for the combination with a diet containing monofloral lupine. Further studies should be conducted under realistic field conditions to evaluate the potential risk on bee health.

2.02.P-Mo074 Development and Validation of a Method by UHPLC-MS/MS in 'Multiresidue' Pesticides From Honey From Bees of the Genus *Tetragonisca angustula*

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The increasing for the demand of more sensitive methods also requires an improvement of the quality of chemical measurements, traceability, and reliability. To provide a safe new analytical method, with trustable results, and precise report of the sample characteristics, it must consider an evaluation called validation. The process of validating an analytical method must pass through the planning and execution of the method, to assure the method is reliable. One of the largest groups of tropical pollinators are the indigenous stingless bees, being the main pollinators of several ecosystem, some of them with a directly dependence of the bees. Seeking increase the production, the use of pesticides is a common measure to eliminate insects that devastates the crops. Whoever, these substances also reach non-target species, mostly pollinators that are gainful for the crop. The Tiamethoxam is an insecticide of second-generation neonicotinoids, largely used in crops in Brazil and worldwide. Mostly found in lettuce, cotton, potato, and bean, are also used in highly pollinator dependent crops like strawberry. Like the other neonicotinoids studied, Imidacloprid and clothianidin, its toxic effects on the pollinating agents have been described in the literature and associated with disorders and collapse of entire population of bees, being harmful to agriculture and endangered species. Abamectin is an avermectin insecticide that acts on the gamma-aminobutyric acid (GABA) receptors in both vertebrates and invertebrates, compromising the nervous system and is also largely used and not selective, reaching bees. The fungicide Difenconazol hasn't direct effect in invertebrates but, like the other four, are associated with Colony Collapse Disorder and must be studied. The present work aimed to determine and validate an analytical method for pesticides detection in honey from the indigenous bees *Tetragonisca angustula* in a way to contribute with studies of chronic effects of the exposure in bees and larvae. The modified QuEChERS extraction method was used to prepare samples, which were then analyzed by UHPLC-MS/MS. The validation parameters method must include a linear range between 0.05 and 0.0100 $\mu\text{g mL}^{-1}$ and precision values greater than 80%, accuracy less than 20% and three-level recovery satisfying the international validation guidelines. The Matrix effect was bellowing the allowed limits for honey. The method can be applied in further studies, becoming a useful tool to determine these pesticides and contribute to environmental risk assessment involving.

2.02.P-Mo075 Historical Control Data (HCD) in Non-Target Arthropod Field Studies: A Way Forward?

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Non-target arthropod (NTA) off-field studies measure biological responses to test item exposure in highly complex natural systems. The settings usually include a control and multiple treatment groups. By design, the controls are not controlled systems as in laboratory studies, but untreated natural groups with a number of biotic and abiotic factors increasing the variability in the system (e.g. soil moisture, humidity, temperature, species interactions). In addition, these factors and their interaction with the NTA communities are not stable but can vary over time. It is critical to distinguish between this background variability and treatment related effects. Therefore, understanding the variability and optimising the statistical precision is critical. Due to several reasons (e.g. field size constraints), it is usually not possible to increase the number of replicates within studies, which would support statistical precision. Historical control data (HCD), which consist of pooled control group responses from previous (comparable) studies could help to distinguish between true treatment related effects from chance findings, as they can represent the extent of background variability (e.g. in abundance of NTA taxa). HCD data could be used to check if a control population, to which the test population is compared, shows a population development as expected from previous studies, or if this particular control population shows some specific features, which could lead to either masking of effects, or the appearance of an effect in the test item group, where indeed no such effect exists. Historical control data (HCD) are used in a variety of contexts already (e.g. mammalian toxicology studies), and recently, Brooks et al (2019) discussed, if and how HCD could be used in higher Tier studies (e.g. mesocosm studies). Here, we investigate, if HCD could be used in NTA off-field studies. We especially focus on the extent of variation in abundance data of NTA between years and if these differences can be explained by selected abiotic factors (e.g. weather data). Our data set comes from a (spatially constrained) meadow landscape in South-western France where NTA were collected over several years.

2.02.P-Mo076 Pyrethrin As a Toxic Reference for Field Studies With Non Target Arthropods (NTA)

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In ecotoxicology, especially terrestrial field studies, the use of a toxic reference substance is a key validation tool. It is required to show that exposure of the non-target arthropod (NTA) community occurred and that the sampling was adequate to show effects.

Historically, pesticides such as organophosphates were the status quo choice to achieve the two goals and to ensure the regulatory validity of the studies. Their toxicity and persistence in the environment resulted in a Europe wide ban in 2020. This brought several challenges to the conduct of NTA studies due to a lack of a good replacement reference-substance. Other insecticides like synthetic pyrethroids, pyridines or insecticides from the ryanoid class have specific mode of actions, and thus, do not affect certain taxonomic groups, such as mites, springtails or Hymenoptera. Considering the importance of the toxic reference, we asked the following questions: Can we find a good replacement reference substance, for the organophosphate insecticides, which is less persistent and still achieves the goals of a toxic reference? To answer the question a field trial started in autumn 2021 in France. The trial was located in a natural meadow and had a randomized block designed with three treatments: water control, Pyrethrin and an organophosphate insecticide were applied. The NTA communities were sampled a few days before the application and at three days, one week, four weeks and six months after the application. The effects of the application of the two types of insecticides will be compared in terms of magnitude and duration of effects on a range of arthropod taxa, typically occurring in off-crop meadow habitats. Based on these results we will discuss the potential of pyrethrin as a more sustainable and environmentally friendly toxic reference in terrestrial ecotoxicology studies.

2.02.P-Mo077 Brood Termination Rate in Honey Bees: Comparison of BTRs Obtained Under Semi-Field Vs Open Full Field Conditions

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The potential impact of pesticides on honey bee (*Apis mellifera* L.) brood is often investigated under higher-tier semi-field, worst-case exposure conditions, according to OECD GD 75, with the brood termination rate (BTR) as one of the key measurement endpoints to be considered. Historical data from such semi-field studies, where brood cells with eggs are marked out and the 7-day exposure period takes place under tunnel conditions, show a high variability in the BTRs within the untreated control groups. In contrast, control BTRs obtained under full-field conditions with free-flying honey bees are substantially lower and less variable. The current analysis by the ICP-PR Bee Brood Working Group investigated the magnitude and variability in BTRs for a negative control and a reference chemical (*i.e.*, fenoxycarb) at two subsequent brood cycles, the first started under semi-field conditions (*i.e.*, while colonies are confined in the tunnels), while the second started under full-field conditions after completion of the first brood cycle when colonies were in the post-exposure monitoring phase of the study and colonies are no longer confined. In addition, the results obtained for the reference chemical fenoxycarb provide insight on the duration of effects on brood caused by an active substance with known insect growth regulating (IGR) properties. These results will be compared and discussed regarding the interpretation of BTRs gathered from bee studies.

2.02.P-Mo078 Honey Bee Brood Studies Under Semi-Field Conditions: Evaluation of Effects Mitigation With the Test Item Application During Evening in Absence of Bee Flight

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Honey bees (*Apis mellifera*) are important pollinators of major agricultural crops around the world. Populations of honey bees have been reported to be in decline in the EU and USA since 1961, and pesticide exposure has been suggested as one of the non-disease factors for this decline. Therefore, regulatory authorities around the world are paying close attention to this issue. A three-tier assessment scheme consisting of laboratory studies, semi-field studies, and field studies is a common approach for evaluating pesticide risks in honey bees. The decision-making scheme used in pesticide assessment in the EU and USA requires that a semi-field test is undertaken if effects on bee brood development cannot be excluded. In the present study the impacts of an insecticide belonging to the pyrethroid family was evaluated in a semi-field test. The application was performed during the full flowering of *Phacelia tanacetifolia* both during daily bee flight and during the evening without bee flight to evaluate the possibility of mitigation of the effects. The OECD No. 75 guidance document with some method improvements (use of bigger colonies, start in spring, use of large tunnels size) were followed with the aim to reduce the variability of the replicates and reducing the Brood Termination Rate (BTR, failure of individual eggs or larvae to develop) in the negative control. A honey bee semi-field test was successfully carried out: at the end of the test the BTR of the negative control was < 30 % and clear adverse effects were observed in the brood development after treatment with the reference item (BTR > 90 %) confirming the sensitivity and reliability of the test method. No statistical difference was observed between the BTRs calculated from the treatment during the flight activity and during evening without flight activity, and respect to the negative control. These results indicated that the test item could not represent a potentially unacceptable risk for honeybee.

2.02.P-Mo079 How to Feed the Mason Bee *Osmia bicornis* -Establishing a New Feeding Technique for the Acute Oral Bioassay With Solitary Bees

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The loss of insect biodiversity in agricultural landscapes during the past decades indicates a need to extend the current risk assessment applied for pesticides from honey bees to other pollinators. One candidate species for establishing a new acute bioassay is the Red Mason Bee *Osmia bicornis*. Currently, an acute oral toxicity test for the solitary living Mason Bee (*Osmia* spp.) is under development by the OECD ring test group. Some of the major challenges observed during the initial trials were low food consumption, low number of feeders and a tendency towards elevated control mortality in the test (? 15 %). Hence, objective of this study was to improve the applied feeding methodologies to increase the acceptability of the treated feeding solution to the

test organisms and to reduce the control mortality. The experimental set-up of the ring test group was used introducing the single bees after 24 hours starvation in queen cages with queen cups containing sucrose solution. The weight of the queen cup was determined before and after exposure to assess the ingested amount of feeding solution. In a first trial, balls with different colours were added to the queen cups. The feeding solution was added afterwards. The introduction of coloured balls in the queen cups increased the relative amount of feeders up to 20 % compared to the queen cups containing only feeding solution. In a second trial, an adaption period was included to enable the mason bees to learn from each other how to feed from the queen cups ('advanced feeders'). The bees were held together in groups of the same sex beforehand and were fed the same way as in the following application. The number of feeders in the 'advanced feeder' group was 13 % higher compared to the 'first time feeder' group. Taking these measures a series of acute oral tests with *Osmia bicornis* resulting in low control mortalities (0 to 11 %) and representative amounts of feeders (54 – 90 %) were conducted. Conclusively, an improved feeding methodology and the inclusion of an adaptation period provided evidence that the challenges of the low food consumption could be solved, although further experiments should be conducted to confirm the inter-laboratory reproducibility of the results

2.02.P-Mo080 Interactive Effects of Agrochemicals on Survival of the Red Mason Bee (*Osmia bicornis*)

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In agricultural landscapes, bees may be exposed to many plant protection products applied to crops that are attractive to pollinators. Apart from direct effects of individual active ingredients or their formulations actually used in the field, which are evaluated following legal requirements, agrochemicals applied in mixtures or sprayed consecutively in short time intervals may potentially interact with each other, causing unpredictable effects on bees. The mixture effects of agrochemicals are, however, poorly recognized, especially for non-*Apis* bees which, in contrast to honeybees, are not included in any standard procedures for ecological risk assessment of pesticides but may respond differently because of differences in physiology and ecology. We tested the impact of the following agrochemical formulations on survival of *Osmia bicornis* females: Dursban 480 EC (with the organophosphate chlorpyrifos), Sherpa 100 EC (with the pyrethroid cypermethrin), Mospilan 20 SP (with the neonicotinoid acetamiprid), Karate Zeon 050 CS (with the pyrethroid lambda-cyhalothrin) and Closer (with sulfoxaflor from the sulfoximine class of systemic insecticides). The agrochemicals were applied topically in three binary mixtures using a full factorial design with five concentrations. The concentrations were selected based on literature data for residues of their active substances recorded in the field and were expressed as the Recommended Application Concentrations (RAC). We found a strong negative effect of Dursban 480 EC ($p < 0.001$), with LT50 of 1 day at 0.7 and 1 RAC (LT50=30 days for control), a much weaker but significant effect of Sherpa 100 EC ($p = 0.02$) at 0.25 to 5 RAC (LT50 from 10 to 30 days), and no effect of their interaction on bee survival. In the case of Sherpa 100 EC × Mospilan 20 SP mixture, the effect of Sherpa 100 EC ($p = 0.02$) on bee survival was surprisingly lower with the presence of Mospilan 20 SP ($p = 0.001$ for interactive effect), even at 5 RAC. We also found a negative effect of Closer ($p < 0.001$), which was especially strong at RAC₇₅, and smaller effect of Karate Zeon 050 ($p < 0.001$) (visible mostly at RAC=25) and their interaction ($p < 0.001$) showing that at high Closer concentrations (RAC₇₅) the effect of Karate Zeon 050 on bee survival disappeared due to the high toxicity of Closer. This study was supported by National Science Centre, Poland (UMO-2017/26/D/NZ8/00606) and the EcoStack project (Grant Agreement 773554 - H2020-SFS-2016-2017/H2020-SFS-2017-2).

2.03 Biomonitoring of legacy and emerging contaminants in wildlife (Part I)

2.03.T-01 Quantification of the Sources of Lead Exposure in the Bearded Vultures From the French Alps

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In order to perform a risk assessment of lead ammunition on the conservation of bearded vultures in the Alps, we have estimated the contribution of the different sources and routes of Pb exposure in the bearded vultures, like soil ingestion during soil bathing, soil ingestion during feeding, ingestion of bones and soft tissues of prey and the ingestion of Pb shot pellets and fragments of Pb bullets. We also evaluated the contribution of these sources by the analysis of their stable Pb isotope signatures. Moreover, Pb analysis along sections the feather rachis was used to detect the pulses of abnormal exposures, which together with the ptylochronology may help to estimate the incidence of lead poisoning in the bearded vultures. Based on energetic (food) requirements of bearded vultures, we calculated the Estimated Daily Intake with regard to the mean concentration of Pb detected in food. The concentration of Pb, excluding two outliers in liver values, give mean values of 0.15 ppm for muscle, 0.22 ppm for liver and 0.40 ppm for bone. We may estimate and intake of less than 0.025-0.036 mg/day when feeding on soft tissues and 0.157 mg/day feeding on bones. Pb levels in bathing and feeding sites were relatively low (< 60 ppm), but soils from or around Pb mines showed elevated levels of Pb (10,000-20,000 ppm) that are well above the threshold of highly contaminated soils (>300 ppm) and comparable with polluted sites. Pb isotopic composition differed between primary sources (soil or ammunition), but also among different types of ammunition (bullets). The prey samples with highest Pb levels (potential contamination with bullets) showed a trend towards some of the bullet types and golden eagle samples and faecal samples with high Pb levels also showed lower 208/207 ratios. However, soil samples tend to show higher 208/207 ratio than ammunition (bullets). From feather analysis, we may conclude that the estimated prevalence of lead ammunition ingestion could be 5% and the monthly incidence 2.5%. In principle, as found in other vultures, lead from soil may contribute to the elevation of lead levels in the food chain and in the chronic exposure in the avian scavengers, but the extremely levels in some birds can only be explained by the ingestion of ammunition.

2.03.T-02 First Diclofenac Intoxication in a Wild Avian Scavenger in Europe

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Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) extremely toxic for old world vultures after ingestion of domestic animals tissues previously treated with it. Diclofenac intoxication was responsible of dramatic population declines of three native South Asian *Gyps* vulture species two decades ago. Even so, this NSAID was authorised in 2013 for veterinary use in Spain, which hosts the biggest vulture populations in Europe. Including cinereous vultures (*Aegypius monachus*), which is classified as “near threatened” by the International Union for Conservation of Nature (IUCN). Cinereous vulture has been the target species in reintroduction and monitoring programs developed in Catalonia (NE Spain), achieving a stable population composed by 61 individuals and 15 breeding pairs in 2020. In September 2020, GPS-GSM movements from one tagged fledgling suddenly ceased and the individual was found dead in the nest. Data obtained from GPS device permitted to estimate the time of death, onset of poisoning signs and the potential time and location of diclofenac exposure. Post-mortem examination of the fledgling revealed severe visceral and articular gout, which was confirmed by histopathological findings that indicated that visceral gout was caused by a toxicant. In fact, diclofenac was detected at concentrations of 26.5 ng/g in the liver and 51.4 ng/g in kidney, respectively. Diclofenac levels are in the range of toxic concentrations for *Gyps* vultures, suggesting similar vulnerability of other genus of avian scavengers, such as *Aegypius*. In addition to diclofenac, tissues were subjected to a wider screening for other NSAIDs and chemicals commonly detected in poisoning events in Spain (carbamates, organophosphates...) obtaining negative results. Post-mortem examination findings, together with the toxicological and histopathological results, provide evidence that this individual died of diclofenac intoxication. This is the first case of diclofenac poisoning in Spain (and Europe), and the first report of diclofenac poisoning in cinereous vultures. The detection of one confirmed case of diclofenac intoxication in a closely GPS monitored vulture is of particular concern, due to it could indicate diclofenac intoxication can remain undetected in a high number of birds, underestimating its potential impact in vulture populations. This case report supports the need to closely monitor vulture populations and implement strict regulatory measures to prevent diclofenac poisonings.

2.03.T-03 Effect of Moulting Patterns on the Elimination of Hg in Seabirds' Feathers: Implications for Hg bioMonitoring

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As they occupy high positions in the food webs, seabirds are relevant bioindicators of biomagnifying contaminants such as mercury (Hg), one of the most toxic metal, which is now regulated by the UN Convention of Minamata. Feathers can be non-lethally sampled during the reproduction period, and constitute an interesting tissue to implement Hg biomonitoring programs at large spatial and temporal scales. After its bioaccumulation in the body, Hg is excreted in feathers during their synthesis because of its strong affinity for the sulphur residues of keratins. The elimination of Hg in body feathers depends largely on moulting patterns, which are variable depending on seabird species/group. For example, penguins have a catastrophic moult, with all body feathers being replaced in a highly synchronous manner once a year. Species such as alcids and larids have an asynchronous moult of the whole plumage or parts of it (e.g., nuptial moult). Finally, other species such as albatrosses have a prolonged, continuous moult. By studying Hg body feather concentrations of seabirds from the Arctic and the Southern Ocean, we propose to define different strategies from the “capital seabirds”, which bioaccumulate Hg over long periods to the “income seabirds”, which eliminates the Hg as it is incorporated. Our objective here is to show the fundamental principles which must be considered to use body feathers during Hg biomonitoring studies in the light of recent studies. Indeed, understanding the temporal integration of Hg in feathers is necessary to obtain reliable information for Hg biomonitoring, especially in the context of the Minamata Convention. It is thus crucial to know precisely the biology (moulting patterns) and ecology (distribution range, migration patterns) of seabird species in order to use body feathers for Hg biomonitoring, and identify areas where birds are most exposed.

2.03.T-04 Contaminant-Specific Power Analysis for the Common Buzzard, *Buteo Buteo*, for Cost Effective Monitoring

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Raptors as apex predators are suitable sentinel species for contaminants in the environment. In particular, monitoring of contaminants in raptors may be used to assess the effectiveness of chemical risk mitigation measures and to assess the effectiveness of chemicals regulations more generally in their aim of protecting environmental (and human) health. It is important when interpreting monitoring data or planning such studies that we have an understanding of whether the number of samples used enables us to detect changes in exposure of the desired magnitude. This study used recently generated contaminant data for the common buzzard, *Buteo buteo*, to explore the relationship between the numbers of samples used the power to detect temporal changes in specific contaminants. . To investigate the effects of bulk sampling, the Sum PCB, Sum PBDE, Sum SGARs, total mercury, and lead residues values was used as a reference data set, providing a guide as to the inherent variability of these

concentrations. We simulate the impact that within-year pooling of samples (of 2, 3, 4 or 6 individuals) has on the provision of representative country-scale data on the detection of changes over time in average residue concentrations with a view to assessing the power of monitoring with pooled samples. Power here means the size of change that can be detected with a certain statistical probability over a specified time period. We examine trade-offs between extent of sample pooling, number of pooled samples that are analysed, and projection of the magnitude of change in environmental concentrations that would be detectable if monitoring were continued for 5 and 10 years.

2.03.T-05 Chemical Contamination in the Muscle of Several Fish Marine Species: Comparison Between the Four Regional Areas of the Marine Strategy Framework Directive

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The European Marine Strategy Framework Directive (MSFD) aims to achieve or maintain the “Good Environmental Status” (GES) of European marine waters. To implement efficient monitoring programs, the contamination levels on target substances are required. The main objective of this study is to explore the potential differences of contamination levels among several marine fish collected in the four regional areas covered by the MSFD in France, for several family of inorganic and organic substances. Four economically important fish species (the European hake *Merluccius merluccius*, the whiting *Merlangius merlangus*, the mackerel *Scomber scombrus* and the spotted dogfish *Scyliorhinus canicula*) were sampled during the annual Data Collection Framework surveys, namely CGFS in the English Channel and Celtic Seas in 2018, EVHOE in the Bay of Biscay in 2018 and MEDITS in the Mediterranean Sea (Gulf of Lion and east of Corsica) in 2017. The concentrations of four families of chemical substances including both inorganic contaminants (trace metal elements) and organic contaminants (chlorobiphenyls, dioxin and furans and polybrominated diphenyl ethers), were measured in the muscle tissue of studied species. Variations in contamination levels among species and regional areas were assessed by permutational multivariate analysis of variance, based on distance matrix of contaminants concentrations. In addition, profiles of contamination were performed for chlorobiphenyls and polybrominated diphenyl ethers, according to the number of halogen atoms. According to substances families, variations in species and/or French subregional areas of MSFD have been detected and we will link them with subregion characteristics and fish biology. Preliminary results showed that higher levels of metals, especially in As and Hg, were observed in Mediterranean seas than in Atlantic ones and in spotted dogfish than the other fish species and higher levels of PCB were found in Bay of Biscay and English Channel. However, the fish species presented similar concentrations excepted the spotted dogfish from English Channel and Mediterranean Sea that showed lower PCB concentrations. In addition, PCB profiles were similar among species within the same regional area with the predominance of penta, hexa and heptachlorobiphenyls.

2.03 Biomonitoring of legacy and emerging contaminants in wildlife (Part II)

2.03.T-06 Anticoagulant Rodenticides in Birds of Prey From Western Canada: Exposure and Toxicity

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As the dominant means for control of pest rodent populations globally, anticoagulant rodenticides, particularly the second generation compounds (SGARs), have widely contaminated non-target organisms. Here we present data on hepatic residues of anticoagulants in 748 raptorial birds found dead or brought into rehabilitation centres in British Columbia, Canada, 1988 to 2018. The incidence and extent of exposure varied by species, geographic area, and over time, with at least one SGAR residue detected in 74% of all raptor livers sampled. Hepatic SGAR concentrations varied from < 0.005 to 1.83 mg/g wet wt. By comparison, first generation compounds (FGARs), typified by warfarin, were detected in just 5% of the raptors analyzed. Highest rates of exposure were in larger owl species with diverse diets, that can include targeted rats, and that have inhabited suburban and intensive agricultural habitats, particularly Barred (*Strix varia*) and Great Horned (*Bubo virginianus*) owls. Overall exposure to at least one SGAR in those two species in the study area was 95% and 81%, respectively. Barn owls (*Tyto alba*), mainly a vole (*microtus*) eater, had a lower incidence of exposure of 66%. Concentrations of SGARs were highly variable, for example in Barred owls, the geometric mean total SGAR = 0.12, with a range < 0.005 to 1.81 mg/g wet wt (N = 208). Putatively bird-eating raptors also had relatively high incidence of exposure with Cooper’s hawks (*Accipiter cooperii*) at 75% (n = 36), Sharp-shinned hawks (*Accipiter striatus*) 60% (n = 15), and Peregrine falcons (*Falco peregrinus*) at 75% (n = 8). Analysis of the spatial trends and patterns revealed that exposure of Barred Owls occurred in landscapes with a high proportion of residential land use. Mean concentrations of brodifacoum and difethialone decreased in Barred and Great Horned owls, while bromodialone increased coincident with implementation of regulatory restrictions on their usage commencing in 2012, and which followed a trend among commercial applicators to move from brodifacoum to bromodialone about 2008.

2.03.T-07 Detection of Enrofloxacin and Ciprofloxacin in Domestic Animal Carcasses and Eurasian Griffon Vultures (*Gyps fulvus*) in Spain

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Veterinary pharmaceuticals are commonly used to treat livestock and therefore, residues can remain within their carcasses after

death with potentially risky consequences for scavengers. One example are antibiotics, which have already been detected in plasma of vultures. The presence of quinolones may be used as a contamination indicator to elucidate antibiotic contamination and potential effects in higher vertebrates. Griffon vultures (*Gyps fulvus*) are good sentinels. We present data regarding exposure to quinolones and residues in carrion supplied for their consumption in Aragon, NE Spain. Griffon vultures (n = 656) were captured at 19 feeding sites in Aragon (NE Spain) between 2008 and 2011. Besides, carrion samples from pig (n = 114), sheep (n = 25), cow (n = 2) and goat (n = 1), were taken during supplementary alimentation procedure in 10 feeding sites during 2011, 2017 and 2018 in Aragon and Castellon (close to Aragon border). Selected tissue samples were muscle and liver. Quinolones were detected in plasma from 13% (85/656) griffon vultures captured in Aragon. Enrofloxacin was detected in 11% (n = 73), while ciprofloxacin was detected in 6% (n = 38) and both could be detected in 4% (n = 27). Concentrations found ranged between 0.14 and 36.68 ng/mL for enrofloxacin and between 1 and 66 ng/mL for ciprofloxacin. Prevalence differed between feeding sites, showing up to 66% enrofloxacin and/or ciprofloxacin prevalence in feeding stations sampled in Teruel province. In addition, Pb concentrations detected also differ among feeding sites, suggesting exposure to antibiotics or Pb depending on carrion species ingestion. Enrofloxacin and ciprofloxacin were detected in 18% (26/142) of the total animal tissues supplemented at feeding stations in Aragon, mostly in pig (n = 24). We detected higher average concentrations in liver (1578 ng/g and 355 for enrofloxacin and ciprofloxacin, respectively) than in muscle (642 ng/g and 185 ng/g for enrofloxacin and ciprofloxacin, respectively). Moreover, we used Generalized Linear Mixed Models (GLMM) with multiple environmental variables to explain the presence of antibiotics in vulture plasma, which correlated positively with livestock related activity and negatively with gaming. Low levels of fluoroquinolones were detected in the plasma of griffon vultures, and prevalence varied depending on the feeding site where the vultures were trapped. These results indicate that vultures are regularly being exposed to quinolones through the carrion supplied at feeding sites.

2.03.T-08 Assessment of Per- and Polyfluoroalkyl Substances in an Arctic Seabirds Through Non-Invasive Sampling

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The monitoring of pollutant trends in areas of high ecological relevance, such as the Arctic that is assumed to be a pristine environment, is an increasingly important issue. Areas with lower direct inputs of pollutants than populated areas are subjected to the transport of pollutants through atmospheric and ocean circulation. Emerging pollutants, such as per- and polyfluoroalkyl substances (PFAS), which are present in the composition of a large number of consumer products, may reach remote locations in the Arctic. PFAS are considered ubiquitous pollutants due to their occurrence at global scale in the environment, including biota. PFAS are found in high concentrations in Arctic birds, but their physiological and ecological effects remain almost unknown. The aim of the present study was to measure concentration levels and pollutant trends in the little auk (*Alle alle*), a seabird with an ecology and habitat that have been affected by global change and environmental pollution. The specific objective of this work was to assess the contamination levels of selected PFAS in the plasma of little auk at different life stages, to investigate potential maternal transfer and dietary exposure. Results showed a significant decrease in plasma concentration of total PFAS (?PFAS) was observed between 2018 and 2019 in both life stages of little auk. Linear perfluorooctane sulfonate (L-PFOS) was the main pollutant in seabird plasma, with the highest concentration found in chicks in 2018 (2919±792 ng/g - 27%; mean ± SD - RSD), followed by adults in 2018 (2661±1752 ng/g - 66%). As expected, the PFAS pattern was dominated by perfluorooctane sulfonate (PFOS), followed by even-chain carboxylates. Regarding parental transfer, chicks presented similar values to their corresponding parents from the same nest. Adult males presented greater variability of ?PFAS plasma concentration than either the females or chicks; nevertheless, males and females presented concentrations similar to those founded in chicks. PFAS were detected in all little auk samples collected from East Greenland; L-PFOS (linear and branched isomers) and PFUnDA were predominant. Results indicate decreasing concentrations from 2018 to 2019 in the plasma of those seabirds, which may be attributed to ecological factors (currently under investigation). PFAS levels were similar between adults and chicks, thereby suggesting the predominance of dietary exposure.

2.03.T-09 Comparative Study of Polycyclic Aromatic Hydrocarbons and Alkylphenols in Plasma of Seabird Chick Species From the English Channel

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Coastal ecosystems and particularly the estuaries of large river such as the Seine river, are contaminated by a multitude of chemicals, released by domestic, industrial and agricultural activities. Despite international restrictive regulations, polycyclic aromatic hydrocarbons (PAHs) and alkylphenols (APs) are still widely used or released in the environment and are targeted in the Water Framework Directive list of priority substances and priority hazardous substances. In aquatic ecosystems, long-lived species at the top of food webs, such as seabirds, are particularly relevant for monitoring the extent of trophic contamination by contaminants. The aim of the present study is to describe PAH and AP impregnation in seabirds from the Seine estuary, in comparison to a reference site with limited anthropogenic pressure (Chausey Islands). The role of the dietary ecology (inferred from stable isotopes of ¹³C, ¹⁵N and ³⁴S) was also investigated. Seabird blood samples were collected on chicks of 4 seabird species: Great black-backed gull (n=81), European herring gull (n=72), Lesser black-backed gull (n=25) and Common cormorant (n=12), collected from different sites (Antifer, Le Havre, Ratier Island) in the Seine Estuary (Eastern English Channel) and from the Chausey Islands (Western English Channel), between 2015 and 2017. Over the three years of study, we showed that gull and

cormorant chicks are exposed to both pyrogenic and petrogenic sources of PAHs as well as to APs. PAH signatures are dominated by low molecular weight congeners, reflecting an atmospheric exposure above the city of Le Havre. Feeding habitat seems to have an influence on AP concentrations: the individuals preferentially feeding on the mainland being highly exposed to APs. Correlation with physiological variables will be further investigated to estimate the impacts on the fitness of individuals.

2.03 Biomonitoring of legacy and emerging contaminants in wildlife (Virtual Only)

2.03.V-01 Invasive and Non-Invasive Heavy Metal Pollution Assessments in Wildlife: A Comparative Review

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Pollution by heavy metals and metalloids is a matter of concern in wildlife conservation and a current One Health problem. Using animal species in toxicology assessments (i.e. biomonitoring studies) positively contributes to a better understanding of health and environmental hazards in many aspects. For instance, to find out the most critical geographical regions or to evaluate their specific toxic effect in each organism. While some organs or tissues are ideal to evaluate pathological lesions as a result of the direct contact with these substances, others are more suitable to perform longitudinal studies in animals suspected of suffering from a long-time exposure. In general, non-invasive samples (as hair, feathers or faeces) represent easier ways of obtaining information on heavy metal exposure, but invasive samples (as internal tissues) provide a more complete and detailed perspective of this health problem. Thus, samples (either invasive or non-invasive) must be selected and interpreted according to the species involved, and also with the aims and conditions of the study. This review aims to compare invasive and non-invasive sampling in wildlife studies, providing a brief comparative resume. **This work was funded by National Funds through FCT under the PhD scholarship 2021.04520.BD.**

2.03.V-02 Linking Legacy Organic Contaminants to Maternal Foraging Areas in the Critically Endangered Kemp's Ridley Sea Turtle: Results From Sampling of Salvaged Eggs

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Biomonitoring studies are particularly challenging for wide-ranging, rare species, such as sea turtles. Typically, female sea turtles are only encountered on land during brief windows of nesting. Sea turtle nests, however, are present for longer periods of time and can be easier to access, especially for closely monitored populations such as those of the critically endangered Kemp's ridley sea turtle (*Lepidochelys kempii*) in Texas, USA. Sampling of non-viable eggs may therefore be an effective, non-destructive tool for establishing baseline contaminant concentrations. In addition, because differences in maternal diet are reflected in eggs, chemical profiles of eggs may be useful indicators of where female turtles foraged. In the current study, we are examining legacy organic contaminants such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in eggs of satellite-tracked female Kemp's ridley sea turtles. Thirty-nine turtles that nested in Texas from 2010 to 2013 were tracked after leaving nesting beaches and their foraging areas in the Gulf of Mexico were determined through state-space modeling. Composite samples (n=48) of non-viable eggs salvaged from the nests of these turtles were analyzed by dual-column gas chromatography with electron-capture detection. Preliminary results indicate that concentrations of many analytes were below the limit of quantitation (LOQ). However, detections above the LOQ were frequent for total PCBs, as well as OCPs formerly used in agricultural production (DDT and chlordane constituents) and fire ant control (mirex). Concentrations tended to be highest in eggs from turtles tracked to the northern Gulf of Mexico. These results demonstrate that sea turtles are still being exposed to legacy contamination, and that the presence of organic contaminants in eggs can be used to differentiate foraging areas. These results also support the analysis of non-viable eggs as a useful, non-destructive sampling strategy for biomonitoring studies of rare or endangered sea turtles.

2.03.V-03 Mercury Bioaccumulation in the East Himalayas of China: Elevational Patterns in Soils and Non-Volant Small Mammals

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Mercury (Hg) is a global pollutant. Due to the “grasshopper effect” and “mountain cold-trapping effect” Mercury (Hg), as a global pollutant, its contamination has been documented in environmental compartments of the Himalayan region. However, little research exists regarding to Hg accumulation in terrestrial wildlife, as well as its driving factors. In this study, surface soil and small mammals were collected in the Lebu Valley, East Himalayas of China, in order to measure the uptake of the long-distance transported Hg along an elevational gradient approximately from 2300 to 5000 m a.s.l. The soil Hg concentrations were measured and predicted mostly by vegetation type as well as soil organic matter, while the Hg in hair of small mammals (Muridae and Cricetidae) showed deeply influenced by soil Hg. Notably, combined with the field survey data, soil and hair Hg were both enhanced in low and mid-elevations, which overlapped the distribution ranges of a majority of mammals. Overall, this indicates that Hg contamination in low- and mid-elevations poses a potential threat to the top predators that consuming small mammals directly or indirectly. Furthermore, our data advances the understanding of Hg dynamics in remote, high mountain ecosystems and provides baseline data for biomonitoring for reduction of Hg emission globally.

2.03.V-04 Mercury Content of Guano As a Proxy for Hg Exposure in Bat Colonies Located in Northwestern Italy

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Bats provide important ecosystem services but are subject to several anthropogenic pressures including exposure to potentially toxic trace elements and organic microcontaminants. Species such as *Myotis blythii*, *M. capaccinii*, *M. emarginatus* and *Rhinolophus ferrumequinum* (all mentioned in annexes II and IV of the Habitats Directive 92/43 EC) form colonies in low-altitude sites and are particularly exposed to human-induced pollution (e.g., trace elements and other chemicals). In bat colonies, guano accumulates in large quantities and may be used as a proxy of organisms' exposure to chemicals (non-invasive biomonitoring). Among legacy contaminants, mercury (Hg) is of pivotal interest because of its tendency to biomagnify along the food webs and its possible transfer from aquatic to terrestrial ecosystems, notably through the emergence of insects with an aquatic larval stage. In the present study, samples of guano were collected in sixteen bat colonies located in Piedmont and Aosta Valley (northwestern Italy), freeze-dried, homogenized, and analysed for total Hg content. Thirteen sites are normally used by the previously mentioned species, while three sites host species belonging to the genera *Hypsugo* and *Pipistrellus*. Measured Hg levels ranged from 0.089 to 0.597 mg/kg, with the highest Hg concentration measured in a colony of *M. emarginatus* near a rice field agro-ecosystem. High levels (0.449 ± 0.021 mg/kg) were also observed in a mixed colony (dominated by *M. capaccinii*) roosting at Lake Maggiore, where Hg pollution of industrial origin occurred throughout the twentieth century. Our analyses did not detect preferential Hg accumulation neither for individual bat species nor for specific land uses or foraging habitats. Additional analyses (e.g., diet characterisation via metagenomics approaches) are needed to better understand the relationship between land use and Hg accumulation in different bat species and ecosystems.

2.03.V-05 Occurrence of Current Use Pesticides in Bumblebees and Environmental Matrices Under Different Land Uses Scenarios in the Southeast Pampas, Argentina

Franco Cecchetto¹, Agustina Villalba², Tomás Mac Loughlin³, Damián Marino⁴, Matias Maggi⁵ and Karina Miglioranza⁶, (1)IIMyC, CONICET-UNMDP, Mar del Plata, Argentina, (2)Instituto de Investigaciones Marinas y Costeras (IIMYC) CONICET, UNMDP, Argentina., Argentina, (3)Universidad Nacional de La Plata, Argentina, (4)Centro de Investigaciones del Medio Ambiente, Universidad Nacional de la Plata - CONICET, Argentina., Argentina, (5)Instituto de Investigación en Sanidad, Producción y Ambiente (IIPROSAM), CONICET, UNMDP, Argentina, Argentina, (6)University of Mar Del Plata, Argentina Bees (Apidae) are crucial in global biodiversity, ecosystems stability and crops production. In the last years, they have been threatened by several factors, such as agricultural intensification and excessive use of pesticides. In Argentina, the native bumblebee, *Bombus pauloensis*, is one of the most prevalent species of the genus, and could be useful for pollution biomonitoring. The aim of this study is to assess the impact of land use on the levels of Current Use Pesticides (CUPs) in workers and drones of *B. pauloensis*, flowers and soil from foraging sites near Mar del Plata City. Sampling sites included a conventional agriculture area (A1), an ornamental plants field next to a municipal waste landfill (A2), natural reserve with agroecological agriculture (A3) and urban area (A4), and were carried out during application (P1) and post-application (P2) periods of pesticides. 50 compounds were assessed by QUECHERS-LC-MS/MS. The highest pesticides levels were found in A1 for flowers and soils and general higher levels during P1 was seen in flowers. Ten compounds were detected in flowers, four in soil and one in bumblebees. The ?CUPs in flowers during P1 were found to be 10,70 (A1), 4,93 (A2), 1,55 (A3) and 3,07 ng/g (A4), while in P2 were 6,72 (A1), 1,77 (A2), 4,83 (A3) and 127,57 ng/g (A4). Soils showed ?CUPs levels of 3,00 (A1) and 0,9 ng/g (A3) during P1 (no detections in A2 and A4), while in P2 were 34,50 (A1), 5,13 (A2), 1,67 (A3) and 1,47 ng/g (A4). Metolachlor, Azoxystrobin, Imidacloprid co-occurred in flowers and soil. In flowers, Chlorpyrifos predominated (100% of samples(s.)) with a maximum of 7,90 ng/g (A1-P1) and a minimum of 0.67 ng/g (A3-P2), followed by Azoxystrobin (50% of s.) with a maximum of 0,69 ng/g (A1-P1). Curiously, Atrazine and Flurochloridone were found in A3 during P1, and remarkably, Cypermethrin was identified in A4 (P2) at 123,77 ng/g. In soil, Azoxystrobin prevailed (37.5% of s.) with a maximum of 1,67 ng/g (A3-P2). However, in bumblebees only Dimethoate was found in workers from A2 (P2), thus further research is necessary to re-evaluate pesticides impacts on this pollinator. The pesticide pattern seen in all matrices and periods highlights the impacts that conventional agriculture could have on bumblebee's habitat, which represents a key pollinator for many plant species in the area. To our knowledge, this work represents the first analysis of CUPs bioaccumulation in wild bees and related environmental matrices in Argentina.

2.03.V-06 Potential Adverse Effects of Organochlorines on Caretta CARETTA (Linnaeus, 1785) Hatchling Success: First Assessment in Tuscan Nesting Events (Italy)

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Among the three species of sea turtles that regularly live in the Mediterranean Sea, the loggerhead turtle (*Caretta caretta*, Linnaeus, 1785) is the most abundant and the only one nesting along the Italian coasts. Nesting events are reported in Tuscany only from 2013 where they are monitored from the deposition to the hatching. After hatching, in order to collect biological material such as unhatched eggs, pipped and hatchlings, the chamber nest is open. These samples collected during the digging can give valuable information about genetic, pathological, parasitological, toxicological aspects. In this study, for the first time in Italy, are reported the concentrations and the fingerprints of some chlorinated xenobiotics analysed in the unhatched eggs sampled from four Tuscan nests: Marina di Cecina (Li), Rimigliano (Li), Le Marze (Gr) and Riva del Sole (Gr). The toxicological analyses revealed the presence of HCB, 29 congeners of PCBs and the *op'* and *pp'* forms of DDT, DDE and DDD in all 52 samples (13

eggs for each nest). Even if the mean values of contaminant class in the four nests are different, all samples show the same following pattern: *PCBs*>*DDTs*>*HCB*. Levels of contaminants found in our study are lower than those found in the fewer available studies worldwide and they cannot be correlated to the hatching failure. Despite this, the research of these contaminants is still a priority especially in the Mediterranean basin, because even if they were banned many years ago their levels are still high in the marine biota. This reptile, a very long living species, can be considered an integrator of the pollution of Mediterranean basin and, for this reason, the scientific community suggest to consider *C.caretta* a bioindicator in the Good Environmental Status (GES) of Marine Strategy Framework Directive, not only for Descriptor 1 (Biodiversity) and 10 (Marine Litter), but also for Descriptor 8 named Contaminants

2.03.V-07 Risso's Dolphin (*Grampus griseus*) and Persistent Organic Pollutants (POPs): Preliminary Assessment in Stranded Specimens Along the Italian Coasts

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Both globally and in the Mediterranean Sea, ecotoxicological studies on Risso's dolphin (*Grampus griseus*, Cuvier 1812) are scarce. In general, we can say that there is still little knowledge about this species and, in fact, in the Mediterranean Sea, this cetacean has been classified within the *International Union for Conservation of Nature (IUCN)* as "Data Deficient". To fill these gaps at least in a small part, in this study, toxicological analyses were performed in 20 specimens of *Grampus griseus* stranded along the Italian coasts between 1998 to 2021. In the biological material (blubber, liver, muscle and brain) the presence of some Persistent Organic Pollutants (POPs): hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its metabolites (DDTs) were assessed. Through the biomagnification process, these contaminants can be found in much high levels at the top of the food chain and then, Risso's dolphin that is a top predators of marine food chain. These contaminants were evaluated, both quantitatively and qualitatively. The blubber is the biological material with the higher levels, followed from liver, muscle and brain, confirmed the results found in this species and in other cetaceans in the world. The results were discussed according to sex, age and stranding areas. No statistically significant differences were found between genders, age and stranding areas. The levels of POPs found in these specimens were the highest recorded among this species, except for two specimens stranded in 1995 in Ligurian and Tyrrhenian Sea.

2.03.V-08 Roe Deer Antlers As Monitoring Units to Assess Lead Exposure in a Floodplain Polluted by Historical Mining Activities in the Harz Mountains, Germany

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The Harz Mountains (Germany) are one of the oldest Central European industrial areas, and the pollution resulting from more than 3500 years of mining-related activities reaches far into the Harz foreland. Lead concentrations in hard antlers of 105 adult (? 2 years) roebucks (*Capreolus capreolus*) killed between 1939 and 2018 were analysed to assess lead exposure within the floodplain of the Innerste River, which is known to be strongly influenced by historical mining activities in its upper catchment area within the Harz Mountains. Antlers of 50 roebucks killed during a similar time period (1957 - 2018) in a control area not influenced by this river system were also examined. Antler lead concentrations of roebucks killed within or close to the Innerste floodplain ranged between < 0.17 mg Pb/kg (limit of detection, LoD) and 51.48 mg Pb/kg. Median lead concentration in antlers of roebucks killed within the floodplain was 11.12 mg Pb/kg, compared to 2.26 mg Pb/kg in antlers of bucks killed in the floodplain vicinity ($P < 0.01$). Variability of antler Pb concentrations in bucks killed in the floodplain vicinity (CV = 149.8%) almost doubled that of the bucks killed within the floodplain (CV = 75.1%), indicating a more heterogeneous Pb exposure of the former. Lead concentrations in antlers of roebucks originating from the control area, ranged between values < LoD and 5.56 mg Pb/kg, with a calculated median concentration also below the LoD. Lead isotope ratios of antlers from the Innerste downstream area (²⁰⁶Pb/²⁰⁷Pb: 1.179 - 1.181; ²⁰⁸Pb/²⁰⁶Pb: 2.083 - 2.085) overlapped with those established for hydrothermal vein deposits from the Upper Harz Mountains. Sampling year had no significant effect on antler lead concentrations in the bucks shot within and close to the Innerste floodplain ($F_{(1, 78, 444)} = 0.1402$, $P = 0.748$), which highlights the overriding role of lead from wastes of former mining activities in the Harz Mountains on the exposure situation of resident biota in downstream areas. This study demonstrates the long-lasting impact of the historical metal ore mining, processing, and smelting in the Harz Mountains on lead pollution in floodplains of rivers draining this area and the lead exposure of wild herbivores inhabiting these frequently inundated areas. Furthermore, it highlights the suitability of roe deer antlers for monitoring environmental lead levels and the usefulness of lead isotope signatures in antlers for source apportionment of lead pollution.

2.03.V-09 Thirteen Years After: Dynamics of Trace Metals in Free-Ranging Small Mammals Related to Soil Aging 2006-2019

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In terrestrial ecosystems, persistent contaminants such as trace metals (TMs) are accumulated in soils and can be transferred to wildlife through food webs. The lability of soil TMs is time-dependent, and state changes over time are known as "soil aging." It has been reported that soil aging significantly affects the fractionation of TMs in soils that controls their environmental availability and bioavailability. Nonetheless, no study has dealt with the long-term effects of soil aging on the trend of

bioaccumulation of TMs in wild mammals. Therefore, it remains poorly known whether and how soil aging could be involved in the dynamics of TMs transfer in food webs. Based on long-term monitoring in a metal-contaminated site, we assessed the temporal change in (bio)availability of TMs in soils and the trends of TMs in a small mammal species. In this study, soil sampling was carried out in 2006 and 2019 over a 40 km² study area in the surroundings of a smelter located in northern France (Metaleurop Nord) which ceased operation in 2003. In both years, soil samples were taken in the same 37 woody patches located along a soil contamination gradient. The total concentration and sequential extraction of cadmium (Cd) and lead (Pb) in soils were measured. Wood mice *Apodemus sylvaticus* were sampled in the same woody patches in either or both spring and autumn from 2006 to 2019. The total concentrations of Cd and Pb in their liver and kidneys were measured as a proxy for bioaccumulation of TMs. Our primary analysis showed that the total concentrations of Cd and Pb in soils did not significantly change between 2006 and 2019. However, the most mobile fraction of Pb generally decreased, whereas less mobile fractions of Pb increased in many of the patches. These results suggest that Pb in soils became less available during the period. Concentrations of Pb in the tissues showed a decreasing trend, and this trend was more prominent in high contaminated habitats. Meanwhile, no general temporal trend was observed for Cd in the tissues. Overall, our results indicate that soil aging over 13 years played a role in the dynamics and transfers of metals. Changes in availability of TMs in soils probably modified bioavailability and transfers of metals in diet items of wood mice. Our results have strong implications on risk assessment, remediation strategies, and management of polluted sites, which should be implemented according to the spatial and temporal dynamics of TMs.

2.03.V-10 Use of Road-Killed Owls for Contaminant Monitoring

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In the present study we investigated the exposure of legacy compounds such organochlorine pesticides, PCBs, PAH, and emerging compounds such PFAS, pharmaceuticals, and currently used pesticides in road-killed nocturnal raptors. The purpose of the present study is to evaluate the effectiveness of using road-killed owls as a source of valuable samples to monitor environmental contaminants. We proposed owls as sentinel species to monitor contaminants because of their position in the top of the food chain and because of their likeliness to be found road-killed. Working on road-killed animals could be an effective way to establish monitoring programmes in areas without active sampling activities on raptors to obtain valuable tissues for chemical analysis in a non-invasive way. During a decade, road-killed owls found in Southern Portugal rural area were collected and stored from 2010 until 2019. 47 livers suitable for analysis were obtained from five different owls' species: *Bubo bubo*, *Asio otus*, *Athene noctua*, *Strix aluco*, and *Tyto alba*. Necropsies were practiced obtaining liver samples and individuals were sexed and aged. Liver samples were analysed for a total of 91 target compounds. The analytical procedures consisted in three different extraction methods and three different analytical methods based on liquid and gas chromatography coupled with mass spectrometry detection. The results showed the exposure of 20 persistent compounds including PAH, PCBs, and Organochlorine pesticides in all five species analysed. However, residues of emerging compounds were not detected in any sample. Organochlorine pesticides were the most prevalent group of compounds, specially 4-4'DDE present at high concentration levels in all analysed individuals. Age was found as an important factor to take into consideration for POPs bioaccumulation. Meanwhile no differences were observed between sex of individuals. Among the species, *Bubo bubo* and *Asio otus* presented high concentrations levels of legacy contaminants indicating their effectiveness as sentinel species. However, these species were not frequently found road-killed, so other species as *Strix aluco* could be more suitable to establish a monitoring scheme based on road-killed owls. Finally, the multi-contaminant monitoring is proposed to assess the impact of emerging compounds in livers from dead animals found in more anthropogenic landscapes.

2.03 Biomonitoring of legacy and emerging contaminants in wildlife (Poster)

2.03.P-Mo082 Assessment of Exposure to Anticoagulant Rodenticides in Wild Mesocarnivores Linked to Freshwater Ecosystems of the Region of Murcia (Spain) Using Faeces As Alternative Samples

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In spite of EU restrictions for the use of anticoagulant rodenticides (ARs), they still may pose a risk to non-target wildlife, either by the consumption of baits, but also indirectly by ingestion of exposed rodents. This study aims to evaluate the exposure to ARs in different mesocarnivores inhabiting freshwater systems in the Region of Murcia, as well as the factors that determine such exposure by collecting faeces as a non-invasive sampling method. For this purpose, 36 samples were collected in different localities from the following species: Eurasian otter (*Lutra lutra*), marten (*Martes foina*), fox (*Vulpes vulpes*) and genet (*Genetta genetta*). Different variables were categorised according to the land use of each habitat (forest, agricultural or peri-urban) and whether there were extraordinary sources of pollution such as wastewater treatment plants, agricultural activity, or the presence of artificial water reservoirs. Sample analysis was performed by HPLC-QTOF-MS after extraction using acetone. All ARs were detected, being coumatetralyl and difenacoum (25%) the most frequent, possibly due to their high persistence and the fact that they are mainly excreted via faeces. Warfarin and flocoumafén showed significant differences between species, being more frequently detected in genets. Genet was the species with the highest frequency of residue detection (75%), followed by marten and otter (47.06% and 44.44% respectively) and fox (33.33%). On the other hand, the fox which has great ecological plasticity showed the highest concentrations of brodifacoum, a widely used compound. The otter is the species in which the greatest variability of 6 compounds has been detected, probably due to their ecological habits. Coumatetralyl and coumafuryl were the

most commonly detected compounds in the agricultural habitat. The use of coumatetralyl is possibly associated with the existence of moles in home gardens. In conclusion, further studies are needed to know what causes the detection of warfarin in genet faeces and it is suggested that this non-invasive sampling method is a suitable tool for the detection of ARs residues. In addition, exposure is found to be influenced by factors such as interspecific feeding variation and habitat type. On the other hand, risk mitigation strategies are needed for application in all habitat types. Acknowledgements: Funded by Seneca Foundation (20945/PI/18). SE supported by IJCI-2017-34653. LS supported by 21413/FPI/20. Fundación Séneca.

2.03.P-Mo083 Occurrence of Per- and Polyfluoroalkyl Substances (PFAS) in Baikal Seals

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The Baikal seal (*Pusa sibirica*) is an endemic freshwater seal species in Lake Baikal, Russia. As a top predator, the Baikal seal may be vulnerable to biomagnification of persistent organic contaminants. PFAS have proven to be bioaccumulative and associated with immunotoxic effects. Plasma, liver, brain and blubber from 18 Baikal seals (16 pups and 2 adult females, sampled 2011) were analysed for 36 PFAS using a rapid Hybrid[®]SPE extraction technique followed by ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) analysis. A total of 10 different PFAS were quantified across all matrices. Concentrations of PFAS (PFAS) in seal plasma were in the range of 10.1 - 88.7 ng/g ww (median; 25th - 75th percentile: 38.1; 28.9 - 39.5 ng/g ww) with PUnA and PFOS as the dominant PFAS with median concentrations of 11.1 and 8.52 ng/g ww, respectively. Hepatic concentrations of PFAS were in the range of 17.0 - 112 ng/g ww (53.1; 38.0 - 69.4 ng/g ww), similarly PFOS and PUnA showed highest median concentration in liver at 17.3 and 11.3 ng/g ww, respectively. Concentrations of PFAS in seal brain were in the ranges of 58 - 210 ng/g ww (65.7; 89.5 - 97.2 ng/g ww), and similarly PFOS and PFNA were detected at highest levels with median concentrations of 27.6 and 27.2 ng/g ww. However, detection rates were low for these compounds (less than 10% of individuals) and when considering only compounds that were detected in >50% of individuals, perfluorotetradecanoic acid (PFTDA), and PUnA were detected in highest median concentration at 6.61 and 1.85 ng/g ww, respectively. The lowest number of PFAS were detected in blubber with concentrations in the range of 25.8 - 37.5 ng/g ww (31.0; 28.1 - 33.6 ng/g ww) with 7H-Dodecafluoroheptanoic acid (PFHeA) and perfluoro-1-octanesulfonamidoacetic acid (FOSAA) showing highest median concentrations at 15.9 and 7.02 ng/g ww, respectively. PFAS concentrations were present in a similar magnitude of concentration regardless of sex or age (adult and pups). Levels of PFAS in this study were similar to those quantified in Baikal seals previously (sampled 2005) and other pinnipeds, with the exception of PFOS which was lower than reported in other pinnipeds. Geographic differences are likely attributed to different sources of local pollution. To our knowledge, this is the first study to quantify perfluorotridecanoic acid (PFTriDA), PFTDA and perfluoro-3,7-dimethyloctanoic acid (P37DMoA) in Baikal seals.

2.03.P-Mo084 Application of a Tiered Green Toxicology Approach During the Development of Alternative Surfactants

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For decades, large quantities of chemicals have been released into aquatic environments, leading to long-term damage to aquatic ecosystems and biota. These chemicals include not only known toxic substances, but also supposedly harmless compounds, so-called emerging pollutants, whose potential impacts on the environment have long been insufficiently studied and potentially underestimated. Emerging pollutants, used by everyday consumers (detergents, personal care and pharmaceuticals), can enter the environment both due to improper use and disposal as well as due to inadequate purification steps in wastewater treatment plants. In order to limit the development, production and use of emerging pollutants the so called Green Toxicology concept was developed. The aim of Green Toxicology is to identify potentially toxic products early during the development of new products and therefore shifting production and use towards products that are less toxic. In this context, Green Toxicology is essential for the identification of harmful substances and the development of environmental friendly alternatives ("benign by design"), but additionally enables resource- and cost-efficient development ("fail early, fail cheap"). Here, we apply Green Toxicology to the prototype study DreamResourceConti (DRC). DRC is a joint project between chemical industry and academia that aims to develop environmental friendly surface-active substances (surfactants) using CO₂ as a substitutional compound within the polymeric chain and replacing fossil raw materials. For DRC we developed a tiered approach that increases in complexity and selects possible prototype substances based on their environmental impact. This approach separates Green Toxicology into 3 phases: (1) Biodegradability and mechanism-specific screening (endocrine disruption, mutagenicity), (2) acute toxicity screening (algae, daphnia, fish) and (3) chronic toxicity screening (reproduction toxicity) and biomarker assessment (oxidative stress, multi-xenobiotic resistance). Phase 1 and 2 follow the proposed framework of the EU project SOLUTIONS (NORMAN bioassay battery). Based on the successful application and feasibility, we propose the tiered Green Toxicology approach as a novel tool for the development of sustainable and environmental friendly products.

2.03.P-Mo085 Chemical Analysis of Legacy and Emerging Pollutants in the Emblematic Arctic Bird, Ivory Gull

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Pollutant trends are changing in the Arctic due to the sea ice shrinking, which causes a new threat to biota in this area. Some Arctic birds are closely linked with sea ice cover and have suffered significant global population loss. Therefore, they represent key organisms for understanding current impacts on the Arctic ecosystem. The present study is part of a larger project to assess the impacts of Global Change on an emblematic Arctic bird, the Ivory Gull (*Pagophila eburnea*). The specific objective of this work was to investigate the levels of legacy (polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs)) and emerging (per- and polyfluoroalkyl substances (PFAS)) pollutants in the plasma of Ivory Gulls. Fieldwork was carried out in 2018 and 2019 in northeastern Greenland (81.60 °N, 15.57 °W). A total of 22 individuals were caught in July 2018 (n=6) and July 2019 (n=16) and blood was sampled from the brachial vein on each bird. After collection, blood samples were immediately centrifuged for plasma acquisition. For PCBs/OCPs, analyses were performed on relatively small plasma amounts (100 µL) by solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). In the case of PFAS (25 µL), we used online SPE coupled to high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Results indicated that OCPs were the most abundant compounds in bird plasma in 2018 (?OCPs = 209±180 ng/g) followed by ?PCBs (160±118 ng/g). In 2019, concentrations were similar: 97±37 ng/g for ?OCPs and 103±32 ng/g for ?PCBs. Of the 7 and 13 target PCBs/OCPs compounds, 5 and 8 were detected, respectively (limits of quantification in the range of 0.05 to 1.61 ng/g). The most abundant OCP was 4,4'DDE, accounting for 87% and 93% of ?OCPs in 2018 and 2019, respectively. CB-153 was the most abundant PCB, i.e. 46% in 2018 and 52% of ?PCBs present in 2019. In contrast to the results observed for PCBs and OCPs, the mean concentration of total PFAS (?PFAS) in plasma increased over time from 45±27 ng/g in 2018 to 97±35 ng/g in 2019. Out of the 17 target PFAS, 14 were detected (LOQ: 0.003 to 2.756 ng/g). The molecular pattern was dominated by PFOS and an increase of PFTTrDA concentration was observed between 2018 and 2019 from 2.5% to 7.9%. In summary, we showed the widespread occurrence of organohalogens in this Ivory Gull population. Preliminary results may suggest time trends that should be further investigated on a larger number of samples.

2.03.P-Mo086 Organochlorine Pesticides in Blood of Wild and Captive African Leopards, *Panthera Pardus Pardus*

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Compared to aquatic ecosystems, limited information exists on organochlorine accumulation in terrestrial ecosystems and this is specifically true for terrestrial carnivores that have received limited attention in terms of studies on pollutant bioaccumulation. The African Leopard, *Panthera pardus pardus* (Linnaeus, 1758), is a popular focal species for research by ethologists and ecologists, but a noticeable knowledge gap exists with regards to toxicological aspects. To address this gap, the aim of this study was to determine baseline organochlorine pesticide (OCP) concentrations in blood of live wild and captive leopards in South Africa, and to explore the relationship between OCP levels and different conservation management strategies. Peripheral blood samples of captive and wild leopards, representing regular and melanistic individuals within the captive population, were collected while under sedation. The ΣOCP concentrations in blood serum were detected by means of GCECD. Statistical relationships among pesticide content in leopards from three geographical areas, different sexes, age groups and conservation status were examined. Captive leopards from this study had a slightly higher mean ΣOCP concentration (901 ng.ml⁻¹) than wild leopards (768 ng.ml⁻¹), and captive females had lower mean levels of ΣOCPs (797 ng.ml⁻¹) than males (1058 ng.ml⁻¹). Melanistic leopards had higher mean ΣOCP concentrations (1038 ng.ml⁻¹) than their regular counterparts (810 ng.ml⁻¹) and adult individuals had higher concentrations of ΣOCPs (856 ng.ml⁻¹) than sub adults (838 ng.ml⁻¹). OCPs accumulated in the following order DDTs (27%) > HCHs (21%) > Heptachlors (15%) > CHLs (15%) > Drins (14%) > HCBs (8%). Differences in OCP composition profiles of resampled captive individuals were also found. This is the first report on OCP concentrations from leopards in Africa and highlights the need for this parameter to be considered in terms of the conservation management of healthy populations.

2.03.P-Mo088 Temporal Trends of Pollutants in Black Guillemot From the Faroe Islands

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Contaminants such as persistent organic pollutants (POPs) and mercury (Hg) are transported to the Arctic and sub-arctic area with long-range transport, and high concentrations can accumulate in biota. Seabirds can be used as a monitoring organism for pollution since they are relatively high in the food chain and high concentrations of contaminants have been reported in seabirds. Environmental pollutants have been monitored in Black guillemot (*Cephus grylle*) on the Faroe Islands in connection with the Arctic Monitoring and Assessment Programme (AMAP) for the last couple of decades. The results from the monitoring studies can be used for analyses of time-trends for these contaminants in the area. Temporal trends of Hg in liver, feather and eggs, and PCBs and chlorinated pesticides in eggs of Black guillemot were analysed, and the possible influence of a changing diet was measured by analysis of naturally occurring stable isotopes of nitrogen (¹⁵N/¹⁴N). The time trends in black guillemots generally showed an increasing temporal trend for mercury in birds and eggs and a decreasing or non-changing trend for POPs in eggs from the Faroe Islands, indicating an increased deposition of Hg in the area, whereas the deposition of POPs is decreasing or stationary.

2.03.P-Mo089 Spatial Differences in Toxic Elements in Svalbard Reindeer Faeces From Central Spitsbergen, Svalbard
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The Arctic is considered an accumulation zone of long-range, oceanic, and atmospherically transported pollution. In addition, local anthropogenic activities could further contribute to the regional pollution level. The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) is considered an ideal organism to use for monitoring studies in the Arctic. They are key components in Arctic terrestrial food webs and non-migratory, meaning that they reflect local pollution levels. Faeces have in particular been used as non-invasive means of biomonitoring as it reflects the diet of the reindeer, and thus their potential exposure to pollutants. This study aimed to compare the elemental levels and composition in reindeer faeces between two separate sites in central Spitsbergen, Svalbard, to assess the possible contribution of local anthropogenic pollution to the overall elemental concentrations, while also evaluating reindeer dietary elemental exposure. Elemental analysis was carried out (using HR-ICP-MS) in 97 faecal samples collected from Kapp Linné and Adventdalen (n=32 and n=65, respectively). Kapp Linné represents a pristine location, far away from local pollution sources, while there are more human activities (e.g., coal power plant and vehicles) occurring in areas around Adventdalen. The overall elemental composition in the faecal samples differed significantly between the two studied locations. Levels of copper, iron, zinc, and aluminium were higher in the faecal samples from Adventdalen ($p < 0.0001$), while concentrations of the non-essential elements lead (Pb) and cadmium (Cd) were higher at Kapp Linné ($p < 0.005$). Mercury (Hg) levels did not differ significantly between the two sites but showed tendencies to correlate with levels of selenium. The results indicated that Cd levels increased with increasing distance to the ocean (in Kapp Linné), while levels of Hg and Pb correlated with the snow depth (i.e., precipitation) at the location where the excrements were sampled. These results support that oceanic input and atmospheric deposition are important pathways for certain elements to reach Arctic terrestrial ecosystems. It further suggests that reindeer are exposed to a range of toxic elements through their diet. We can however not conclude whether these elements are absorbed in the gastrointestinal tract and distributed to other tissues based on solely faecal samples.

2.03.P-Mo090 Trace Element Distribution Within and Across Ungulate Species and the Potential of Hair As Non-Invasive Long-Term Biomarkers of Wildlife Health

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Monitoring the health and trends of wildlife populations is an essential component of informed conservation management and can serve as an early indicator of ecosystem response to rapid environmental changes induced by e.g. climate or anthropogenic perturbations. However, detailed monitoring in remote areas such as the high-Arctic can be logistically and financially challenging. Additionally, the use of traditional sampling methods to infer wildlife health (i.e. collection of liver or serum samples) are often constrained as they require invasive approaches or rely on hunted animals or carcasses (either of which is impractical for remote, endangered, or declining populations). As such, there is a need for developing novel, non-invasive methods to serve as an indicator of individual health and population demographics/dynamics. While the use of hair/wool as a non-invasive monitoring tool to evaluate wildlife health certainly has potential, knowledge of the within individual chemical distribution, i.e. the distribution and coherence in concentrations of essential and non-essential elements in various tissues (e.g. blood, liver, muscle, spleen, hair) and its linkages to the overall health status of the animal are still largely unexplored. A better understanding of the linkages between trace elements in hair, internal tissues, and wildlife health is therefore needed before this non-invasive diagnostic tool can be used reliably to monitor the health status of wildlife populations. Drawing on iCap TQ-ICP-MS data from different ungulates; muskoxen (*Ovibos moschatus*), Svalbard reindeer (*Rangifer tarandus platyrhynchus*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*), we will present preliminary results on the trace element composition (e.g. Se, Cu, Zn, Mo, Pb, Cd, Hg) in hair and how this links to other internal tissues (e.g. blood) as well as to the general health status of the animal. By including and comparing results from a range of species and locations (i.e. Greenland, Svalbard and Denmark) our results will also form the foundation of a much-needed reference data base on the range of chemical composition in hair and wool from ungulate species. The end-goal of the project and this presentation is to assess the use of hair and wool as a novel, non-invasive long-term tool for monitoring the status and trends of wildlife populations, which in turn can inform and help shape wildlife management practices, especially in remote and logistically challenging areas such as the high-Arctic.

2.03.P-Mo091 Differences in Metal Compositions and Concentrations of Sympatric Predatory Fish and Squid From the South Atlantic Ocean

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Organisms at higher trophic levels may contain metals at elevated concentrations due to accumulation from various sources,

potentially making them more susceptible to detrimental effects, as well as passing them on to consumers. The concentrations of thirty metals were quantified in hake (*Merluccius capensis*), kingklip (*Genypterus capensis*), monkfish (*Lophuis vomerinus*) and chokka (*Loligo reynaudii*) from the South Atlantic Ocean of South Africa, using inductively-coupled plasma mass spectrometry. Nekto-benthic chokka differed significantly ($p < 0.05$) in metal concentrations and composition compared with the three demersal fish predators (hake, kingklip, and monkfish). Demersal fish metal concentrations and relative pattern compositions (fingerprints) were similar. Since the samples were collected within an 80 km radius, the differences are likely due to a combination of factors such as diet, habitat (depth), and differences in the physiological regulation of metals between cephalopods and fish rather than location. This study is the second of its kind, providing information on commercially important marine species of South Africa. Based on South African estimated daily intake, total hazard quotient, and European Union limits for Hg, Cd, and Pb, these four economically important species from the South Atlantic Ocean are safe for human consumption.

2.03.P-Mo092 A Multi-Tissue Investigation for Levels and Trophic Transfer Potential of Toxic Trace Elements in Cattle Egret (*Bubulcus ibis*)

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Exposure to toxic trace elements in birds has become a matter of concern as they transfer through food chain and bioaccumulate in varying concentrations in different tissues posing serious health risks. Therefore, current study is designed to investigate to compare tendency of toxic trace elements viz. Cd, Cr, Pb, Cu, As, Hg to bioaccumulate in liver, kidney, blood, pelvic and pectoral muscles of Cattle Egret (*Bubulcus ibis*). Further, the trophic transfer potential (TTF) of these elements was also evaluated using prey/food samples of Cattle Egret. All of the selected trace elements except Hg and As were detected with varying concentrations in different tissues of Cattle Egret. In general, toxic trace elements followed the pattern as $Cu > Pb > Cd > Cr$. The maximum mean (Min-max) concentrations ($\mu\text{g/g ww}$) of Cu 0.58 (0.11-0.93) and Pb 0.46 (0.40-0.54) were recorded in blood. Whereas highest mean (min-max) concentrations ($\mu\text{g/g ww}$) of Pb 0.46 (0.40-0.54) was observed in liver. The greatest equal mean (min-max) concentration ($\mu\text{g/g ww}$) of Cr 0.17 (0.09-0.25), 0.17 (0.12-0.21) were recorded in pectoral muscle and liver, respectively. Blood and liver were found as the most contaminated tissues followed by kidney, pectoral and pelvic muscles. Differences of trace elements was found non-significant (all $P > 0.05$) among tissues of Cattle Egret reflecting their homogenous distribution in body. Principal component analysis (PCA) corroborated no strong association of any elements with any of the tissue. All the metals except Cr showed considerable higher tendency of trophic transfer potential through food chain in all the tissues of Cattle Egret ($TTF > 1$). The constant exposure of toxic trace elements which have potential to transfer through the food chain can adversely affect the already declining birds population throughout the globe.

2.03.P-Mo093 Bioaccumulation and Effects of Selenium From Surface Coal Mining on an Aquatic Songbird

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In birds, Selenium can induce non-reproductive chronic effects, impaired productivity through decreased fertility, and reduced egg hatchability and fatal embryo deformity. Here we examine the impacts on the American Dipper (*Cinclus mexicanus*) in fast-flowing mountain streams within the Elk Valley, BC, where the coal mining industry poses a variety of long-lasting impacts, including Selenium contamination, to surrounding aquatic ecosystems. The Elk Valley in southeastern British Columbia which is currently host to five active open-pit coal mines and there are plans for substantial expansion. To minimize adverse effects in aquatic organisms, the British Columbia Ministry of Environment has set a selenium concentration guideline in the water column at 2?g/L. In 2016, water directly downstream of active mines, in their standard operation, shows high levels of selenium up to 39.8?g/L. However, the water concentration guideline is inadequate to quantify risk to the ecosystem, as it is greatly complicated by wide variation in biological uptake and selenium speciation. We describe the reproductive status and contaminant levels in American Dippers in this system in the context of risk assessments for these birds and other species here and elsewhere.

2.04 Bringing together exposure and effects: towards a mechanistic understanding of the environmental risk of chemicals in aquatic ecosystems

2.04.T-01 Does Temperature Influence Pesticides' Toxicokinetics and Toxicodynamics Alike? An Investigation With the Full-Guts Model for *Gammarus pulex*

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As global climate change continues, environmental risk assessment (ERA) needs to consider the potential influence on pesticides' toxicity by temperature. However, the underlying physiological mechanisms of temperature effect on the toxicity of pesticides are poorly understood. To address this knowledge gap, mechanistic or process-based effect models based on toxicokinetic (TK) and toxicodynamic (TD) processes, like the general unified threshold model of survival (GUTS), are powerful tools. However, temperature is not integrated into GUTS models per default, limiting its application in ERA for future climates. Thus, we investigated how to consider temperature in GUTS models for pesticides explicitly. For this, we examined the chronic toxicity of imidacloprid (IMI) and flupyradifurone (FPF) and their TK in the freshwater shredder *Gammarus pulex* at different temperatures. With the results of these experiments, we performed a modeling study to investigate the influence of temperature on the TK and

TD of FPF and IMI in *G. pulex*. Model calibrations for the GUTS?FULL models were done in two configurations. In the first, we only corrected the TK parameter for temperature with the Arrhenius equation. In the second, both TK and TD parameters were corrected with the Arrhenius equation. Finally, we compared the performance of the models by using Akaike's information criterion. We found that model calibrations of both model configurations resulted in a good fit of the survival data of *G. pulex*. However, the second configuration performed significantly better than the first. Furthermore, the Arrhenius temperatures for TK and TD parameters differed for both chemicals. Thus, our results demonstrate that GUTS models perform better when temperature is considered for both TK and TD parameters and that both processes are affected by temperature differently. This study indicates that applying mechanistic effect models to experimental data gains insights beyond the standard toxicity information, i.e., which model processes are affected by temperature. Furthermore, explicitly accounting for temperature in GUTS models will reduce uncertainties in its application for ERA and allow a temperature neutral comparison for various contaminant's TKTD parameters between species.

2.04.T-02 Speed It up: How Temperature Drives Toxicokinetic Processes in Aquatic Invertebrates

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Studies for the environmental risk assessment of chemicals are generally conducted under highly standardised laboratory conditions. However, environmental parameters and external stressors are known to exert a strong influence on organism fitness. The ongoing climate change draws a lot of attention towards the parameter temperature. Many studies found a higher sensitivity of aquatic invertebrates towards contaminants with increasing or dynamic exposure temperature. However, the exact mechanisms do remain unravelled. Few studies have been carried out to achieve a more structured and in depth understanding of this link. This study aimed to investigate the influence of temperature on toxicokinetic parameters in *Gammarus pulex* and *Hyalella azteca*. Bioconcentration experiments at four different temperatures (6, 11, 16 and 21°C) with a mix of 12 compounds were carried out using the two amphipod species. Tissue and medium samples were taken in regular intervals and analysed by online-SPE LC-HRMS/MS. From these data toxicokinetic rate constants were modelled and analysed in dependence of the exposure temperature using the Arrhenius equation. A positive relationship between uptake, elimination and biotransformation rates versus temperature was observed. Nevertheless, the kinetic bioconcentration factors (BCFs) of the compounds were generally similar between the different temperatures due to a similar Arrhenius temperature of the uptake and elimination rate constants. However, in some cases BCFs were decreasing towards higher temperatures. The later observation could potentially be explained by the influence of temperature on biotransformation rates, which was supported by high biotransformation product concentration. The present study demonstrated that temperature can be an important driver of toxicokinetic processes and provides data and methods for risk assessment implications. The obtained results help understanding the mechanisms of temperature on chemical uptake, biotransformation and elimination and their interaction resulting in different sensitivity towards chemicals. Further, generated toxicokinetic rate constants can support modelling of environmental exposure as well as toxicodynamic laboratory studies under different temperature conditions.

2.04.T-03 Developmental Insecticide Exposure of Zebrafish Induces Transient Locomotor Effects Mediated by Neuromuscular Alterations

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Among a variety of different modes of action, several classes of insecticides target the cholinergic system. Cholinergic signalling plays an essential role in critical developmental processes of the nervous system such as axon development or synapse formation. Thus, early developmental stages of organisms are particularly vulnerable to chemical exposure. Considering the high number of chemicals with neurotoxic potential, there is an urgent need for new developmental neurotoxicity test strategies involving alternative methods. One such method is the zebrafish light-dark transition (LDT) test, which measures the larvae's locomotor reaction to light changes. Although the LDT is widely applied, it is not yet fully understood whether detected alterations in locomotion reflect structural changes of the nervous system and how persistent these alterations are. This study investigated insecticide-induced locomotion effects in zebrafish and underlying structural changes in neuromuscular components required for locomotion. Therefore, locomotor behaviour of zebrafish larvae was measured after 6-day exposure to non-toxic insecticide concentrations. Additionally, heart rate and body length of each larva was determined. To analyse effects on motor axons and neuromuscular junctions, fluorescence labelling was applied. Reversibility of effects was assessed by exposing zebrafish for 6 days followed by a 3-day depuration phase in chemical-free medium. To monitor the recovery process, locomotion was measured daily during depuration, while body length and heart rate were measured only on day 9. Exposure to the tested organophosphate, carbamate, and neonicotinoid insecticides led to reduction in locomotion, while no effect was observed for the pyridine-derivates. The other tested endpoints showed a different effect pattern for almost all insecticides, while strong locomotor effects could be linked to defects in muscle fibres. The recovery experiment showed that most insecticide-induced behavioural effects recovered within 24 hours of depuration. However, larvae exposed to methomyl, pirimicarb, and thiacloprid, required more time to recover. The results underline that locomotion is a more sensitive endpoint than structural changes. Overall, by investigating the effects underlying alterations in locomotion, this study helps to improve the mechanistic understanding of insecticide-induced neurobehavioral effects.

2.04.T-04 Interpretation of Aquatic Risks at Landscape Scale Using a CASE Study of Pyrethroid Use in Belgium

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The disregard of spatial-temporal dynamics of both pesticide exposure and organisms is a flaw in the current risk assessment methodologies. Additionally, guidance on how to assess environmental risks at the landscape scale in the regulatory context is lacking. Therefore, a framework is needed to i) link specific protection goals to landscape scale assessment endpoints, and ii) develop a common language among stakeholders. For this, example studies with concrete model outcomes are considered helpful. In this study we present such an example for aquatic organisms, with the final aim to feed into the discussion on the use of landscape scale models in ERA. For the example study, an integrated modular model system (xAquaticRisk) was developed that includes spatially explicit simulation of hydrology, pesticide application, fate, and effect models. The application of the xAquaticRisk landscape model to the Rummen catchment area in Belgium, is presented and risk to aquatic organisms is quantified. The Rummen catchment is dominated by the cultivation of fruit orchards. The use of PPPs in these orchards may impact aquatic communities by spray drift deposition onto water bodies. Spray drift resulting from annual single application of 12.5 g/ha insecticide (K_{oc} 1,024,000 L/kg) was simulated over a 20-year period from 1998-2017. Realistic mitigation measures were simulated, assuming 75% drift reduction and a 10 m buffer strip. The effect module Landscape-GUTS (based on the GUTS TKTD models for survival) simulates a GUTS model in all reaches of the Rummen catchment over the same 20-year period for *Asellus aquaticus*, *Cloeon dipterum*, and *Gammarus pulex*. Outputs from the LGUTS module were used to determine LP50 values (multiplication factor for the exposure profile that results in 50% mortality) per year and per reach.

2.04 Bringing together exposure and effects: towards a mechanistic understanding of the environmental risk of chemicals in aquatic ecosystems (Virtual Only)

2.04.V-01 Effect of WATER Exchange Rate in Spiked-Sediment Toxicity Tests With *Hyaella azteca* on Behavior and Bioaccumulation of Hydrophobic Organic Chemicals

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Spiked-sediment tests are widely used to assess the toxicity of hydrophobic and persistent chemicals to benthic organisms. During the test, overlying water is usually exchanged with clean water to supply oxygen and to remove excretes (e.g., ammonia). The frequency and volume of water exchange vary between laboratories and standardized protocols using the same test species, however, little is known about the effects of water exchange methods on temporal and special concentrations and observed toxicity in spiked-sediment tests. We performed 10-day sediment toxicity tests with the freshwater amphipod *Hyaella azteca* using three polycyclic aromatic hydrocarbons (PAHs; phenanthrene [Phe], pyrene [Pyr], and benzo[a]pyrene [BaP]) as model chemicals at five commonly applied water exchange scenarios. The five scenarios were: (i) static (i.e., no exchange), (ii) 3x/week at 2 volumes per day (i.e., semi-static), (iii) every day at 2 volumes per day, (iv) continuous at 1 volume per day (i.e., flow-through), and (v) continuous at 2 volumes per day. The total dissolved concentrations (C_{diss}) of PAHs in overlying water increased gradually in the static scenario (scenario i), whereas those in the other scenarios decreased at the timing of exchange and remained low as compared to scenario i. The difference in time-averaged C_{diss} in overlying water among scenarios was up to 3.7 times (Phe), 6.8 times (Pyr), and 35 times (BaP), whereas the difference in freely dissolved concentrations (C_{free}) was 1.6 times (Phe), 2.7 times (Pyr), and 1.5 times (BaP) at most. Mechanistic modeling revealed that slow chemical diffusion in the upper sediment could not compensate for the fast chemical outflux by water exchange, resulting in a chemical gradient from pore to overlying water. In contrast to overlying water, C_{diss} and C_{free} of PAHs in pore water did not differ among scenarios. In particular, the difference in C_{free} in pore water was within a factor of 1.5 for all the chemicals tested. The 10-day survival rate was above 70% in all the scenarios and the dry weight of amphipods ranged from 0.06 to 0.09 mg, both of which did not differ among scenarios. In addition, the bioaccumulation of three PAHs did not differ among scenarios; the body concentrations were within a factor of two. These results suggest that water exchange scenarios have a substantial effect on C_{diss} in overlying water but not on other concentrations and thus the bioaccumulation and toxicity of *H. azteca*.

2.04.V-02 Effects of Atrazine on the Breeding Activities and the Antioxidant Enzyme Activities of the Aquatic Snails *Lymnaea natalensis*

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Adoption of modern farming methods has increased drastically the usage of the herbicide atrazine in Zimbabwe. Most farmers including small scale farmers have shifted from manual removal of weeds to spraying of herbicides to effectively kill weeds and promote growth of crops. Literature has shown that only small quantiles of the sprayed herbicide reach target weeds, the majority of the herbicides find their way to natural water bodies via, aerial drifts, and runoffs after heavy rains where they affect non target organisms. We investigated the effects of atrazine on the breeding activities as well as on the antioxidant enzyme activities of the water snail *Lymnaea natalensis*. Groups of 20 snails were exposed to water containing different environmentally relevant concentrations (0?g/L, 3?g/L and 30?g/L) of atrazine. The time taken for the snails to lay eggs and for the eggs to hatch were observed. After 3 months of exposure both adults and juvenile snails were sacrificed and homogenates prepared for analysis of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase GPx and glutathione S-transferase (GST). The reproduction and the hatching of eggs significantly decreased in snails exposed to atrazine ($p < 0.05$). The activities of SOD, CAT, GPx and GST were enhanced (between 1.125 to 8 fold depending on the enzyme) in snails exposed to atrazine compared to enzyme activities in unexposed snails. These results showed that atrazine affected the wellbeing of the snails and the activated antioxidant enzyme activities observed were defense mechanisms in snails used for protection against the antioxidant effects of atrazine.

2.04.V-03 Toxicity of Selenium Nanoparticles on *Poterioochromonas Malhamensis* Algae in Waris-H Culture Medium and Lake Geneva WATER: Effect of Nanoparticle Coating, Dissolution, and Aggregation

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Understanding the algal toxicity of selenium nanoparticles (SeNPs) in aquatic systems by considering SeNPs physicochemical properties and environmental media characteristics is a concern of high importance for the evaluation and prediction of risk assessment. In this study, chitosan (CS) and sodium carboxymethyl cellulose (CMC) coated SeNPs are considered using Lake Geneva water and a Waris-H cell culture medium to investigate the effect of SeNPs on the toxicity of algae *Poterioochromonas malhamensis*, a widespread mixotrophic flagellate. The influence of surface coating, z-average diameters, ζ -potentials, aggregation behavior, ions release, and exposure medium properties on the toxicity of SeNPs to algae *P. malhamensis* was investigated. It is found that SeNPs are 5–10 times more toxic in Lake Geneva water compared to the culture medium, suggesting that the traditional algal tests in Waris-H culture medium currently underestimate the toxicity of NPs in a natural water environment. Despite significant dissolution, it is also found that SeNPs themselves are the toxicity driver, and dissolved ions have only a marginal influence on toxicity. SeNPs diameter is found a minor factor in toxicity. Based on a principal component analysis (PCA) it is found that in Lake Geneva water, the nature of the surface coating (CMC versus CS) is the most influential factor controlling the toxicity of SeNPs. In the culture medium, surface coating, ζ -potential, and aggregation are found to contribute at the same level. These results highlight the importance of considering in details both NPs intrinsic and exposure media properties in the evaluation of NPs biological effects.

2.04 Bringing together exposure and effects: towards a mechanistic understanding of the environmental risk of chemicals in aquatic ecosystems (Poster)

2.04.P-Tu046 Algae TKTD Modelling for Tier 2C Risk Assessment: Ring Testing the Validation Studies Using Flurtamone and the Alga *Raphidocelis subcapitata* (Korshikov, Nygaard, Komárek, Kristiansen & Skulberg 1987)

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For the purpose of validating TKTD modelling of algal growth tests, during 2021 two methods for testing of variable exposure regimes for algal growth tests were subjected to ring-testing using the test item flurtamone and the alga *Raphidocelis subcapitata* (Korshikov). The variable exposure algae growth tests aim for validation and calibration of TKTD models according to the EFSA Scientific opinion on TKTD modelling. Two methodologies were the subject of the laboratory comparison test performed, funded by CropLife Europe: Method A: a flow through method Method B: a semi-static method Nine laboratories from Europe and the United States of America participated in the ring-test: Fraunhofer Institute for Molecular Biology and Applied Ecology*, gaic Research Institute, Innovative Environmental Services (IES) Ltd, Witterswil, Switzerland*, Eurofins EAG Agrosience, Easton, MD*, Cambridge Environmental Assessment (CEA), Bayer AG_Division CropScience*, Scymaris, ECT Ecotoxicology GmbH* and Eurofins Agrosience Services Ecotox GmbH (Europe)*. All nine laboratories undertook the semi-static method, while the six participants, indicated with *, agreed to run the flow-through method. Here we concentrate on the semi-static method. The semi-static method used filtration to separate the algal cells from their growth medium and re-suspend them in fresh medium. Three concentrations were used for two exposure patterns. Each pattern employed two 24-h exposure periods, separated by a 24-h non-exposure period in the case of pattern 1 and by a 48-h non-exposure period for pattern 2. In both patterns, after the final exposure period, the algae were grown for a further 6 days to assess their growth over this period. The tests were initiated in November 2021 and the results for both exposure patterns will be presented. Their implications for TKTD algal growth modelling and potential uses in the aquatic risk assessment using Tier 2C will be discussed.

2.04.P-Tu047 Modelling the Contribution of Metabolites in the Overall Aquatic Toxicity of Imidacloprid

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The Environmental Risk Assessment (ERA) of pesticides is important for aquatic systems because pesticides used in farmland usually end up in the water system and may persist in the environment. However, the concentrations of these pollutants are usually not static because these compounds may be biotransformed by exposed organisms or abiotically degraded. Over time, a mixture consisting of the parent compound and metabolites will be produced, making risk assessment difficult. To understand the toxicity of pesticides and their biotransformation products and predict the effects of such mixtures during pulsed exposure, toxicokinetic-toxicodynamic modelling (TK-TD) is considered a promising tool. The TK-TD model quantifies the time course of internal concentration, which is defined as uptake, elimination, and biotransformation (TK), as well as damage and repair processes leading to toxic effects (TD). Imidacloprid is one of the widely used pesticides and has a delayed effect on various non-targeted species. This delayed or so-called time cumulative effect can be explained by the production of the biologically active metabolite imidacloprid-olefin as it has been demonstrated for two aquatic species, *Cloeon dipterum* and *Gammarus pulex* [1]. However, the exact contribution of the parent compound and the metabolite on the overall toxicity has not been distinguished. This study will use the published toxicity and toxicokinetic data of IMI and IMI-ole in two species to calibrate the parent-metabolite-TK-TD model. First, the TK parameters such as IMI uptake rate, elimination rate, biotransformation rate, and IMI-ole elimination rate are estimated. Next, we will obtain compound-specific TD parameters based on the acute toxicity data set of IMI and IMI-ole. Thirdly, we will use the obtained TK and TD parameters to calibrate the damage caused by IMI-ole by simulating

the effects of the internal concentration of IMI-ole only which is generated during IMI exposure. In addition, the parent-metabolite-TKTD model will be validated by pulse exposure experiments with the same species. The selection of the model fits will be based on the AIC value from the same dataset. Our results will deepen the understanding of the toxicity of pesticides in the context of biotransformation and better predict their effects. 1. Huang, A., et al., *The toxicity and toxicokinetics of imidacloprid and a bioactive metabolite to two aquatic arthropod species*. *Aquat Toxicol*, 2021. **235**: p. 105837.

2.04.P-Tu048 Novel MOLECULAR Tool for Monitoring Aquatic Quality

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Due to industrial processes and (domestic) wastewater discharges all kinds of chemicals enter the environment. This has an effect on the water quality in all types of waterways. The Netherlands is a delta of a total of four international rivers. Rijkswaterstaat is responsible for the aquatic quality in the large rivers, lakes and the coastal areas. In the rivers Meuse and Rhine Rijkswaterstaat has border laboratories for continuous measurements using GC-MS and HPLC-UV which cover volatile and non-volatile, polar and non-polar organic compounds, as well as measurements of metals, nutrients and environmental conditions. Biological monitoring is also performed using an in-situ flow-through daphnid system and an algae flow-cytometer. In case of incidents the potential effect on the ecosystem is assessed by means of chemical analyses and some bioassays. However, this doesn't take into account the aquatic system itself! Therefore a pilot has started in order to assess the applicability of Next Generation Sequencing to monitor the effects on the aquatic ecosystem itself as well as the resilience of the aquatic system in case of incidents. The pilot is being performed at the border laboratory in the Meuse. Each month a water sample is taken and analyzed by means of NGS. This characterizes all known bacteria and archaea and gives a clear overview of the biodiversity. The biodiversity is compared with the results of the chemical analyses and the flow-through bioassays. During the pilot we will gain insight in the annual fluctuations of the biodiversity. In case of a highly increased value of a chemical compound either at the border laboratory or elsewhere in the river, additional samples will be taken in order to assess the effect of the compound on the biodiversity in the water as well as the resilience. On the poster the results of the pilot will be shown. Ing. M.H.A.B. Wagelmans MSc, National Water management Centre, Rijkswaterstaat, the Netherlands Ing. J. Claassen BSc, National Water management Centre, Rijkswaterstaat, the Netherlands A.A. de Vos van Steenwijk MSc, Orvion, the Netherlands

2.04.P-Tu049 How DO You Quality Control the Natural Environment?

Nadine Taylor, Marie Brown, Zoe Leanne Jones, Hanna Samantha Schuster and Adrian Terry, Cambridge Environmental Assessments, United Kingdom

The tiered risk assessment approach for plant protection products (PPPs) for aquatic organisms allows for the increasing complexity of studies, with increased realism of the experimental systems. Laboratory-based, lower-tier studies are easily implemented since all studies are performed to strict guidelines, under controlled conditions and have strict validity criteria to allow for standardised, reliable results and reproducibility of studies and their associated data. However, higher tier studies are purposefully uncontrolled to reflect the natural environment. So, how do you implement quality control processes, and generate reliable, reproducible, and robust data from a natural test system? Here we discuss the challenges involved in controlling the uncontrollable and present our findings, to date, on how we manage mesocosm studies to increase data robustness in the face of such intrinsic variation of the test system. Our approaches cover test design, the importance of pre-application phases and homogeneity, replication, sampling techniques, frequencies and representativity, and improving data robustness.

2.04.P-Tu050 Modified Exposure Studies in Aquatic Risk Assessments: How Useful Are They?

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Modified exposure studies are included as a refinement option (Tier 2C) for aquatic risk assessments for plant protection products (PPPs) under the current guidance (EFSA 2013). They can be a useful refinement option for scenarios where the predicted exposure profile indicates that exposure will not be continuous, and therefore ecotoxicity endpoints based on constant exposure may be over conservative. The overall aim of modified exposure studies is to more closely align the exposure profiles used in the ecotoxicity study with that predicted by the exposure profiles, such that we are assessing the more realistic risk from the proposed use of the PPPs. Although Tier 2C refinements are permitted under the current aquatic guidance, some EU Member States have indicated that they will not readily accept such an approach; for example, the central zone MSs recently concluded that Tier 2C refinements 'should generally not be supported at zonal level, considering that implementation in ERA is complex and linked to high uncertainties'. No further detail is provided regarding what 'complexities' and 'uncertainties' are being referred to. The aim of this poster is review active substance dossiers submitted and evaluated at the EU level in the last 6 years (allowing 2 years implementation of the EFSA (2013) guidance) to determine how many substances have attempted to use a modified exposure approach in the risk assessment and, of those, how many have been successful (and why), and where rejected, what were the reasons provided by regulators. Where concerns have been raised by regulators, we will discuss ways in which these could be addressed, aiming to conclude whether or not there is still a role for modified exposure studies in PPP aquatic risk assessments. We will also consider the usefulness of modified exposure studies beyond Tier 2C refinements e.g. supporting the use of TWA PECsw by demonstrating no latency of effects and linear reciprocity.

2.04.P-Tu051 Protection Goals and Associated Endpoints for Environmental Risk Assessment of Pesticides at Landscape-Scale, As Addressed by Spatial Population Models

hans haveco¹, Wim Beltman², Bas Buddendorf³, Maarten Braakhekke⁴, Louise Wipfler², Sascha Bub⁵, Thorsten Schad⁶ and Andre

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Assessment at landscape scale is considered in Europe as an option to improve the realism and relevance of regulatory Environmental Risk Assessment (ERA) for pesticides. Spatially explicit population models quantifying effects at the spatial population level, are part of the extensive modelling toolbox dealing with all steps from application and fate of pesticides to effects at landscape scale. Population models are used to quantify endpoints allowing evaluation of to what extent protection goals at the population level are met. For landscape level ERA, additional endpoints might be needed, addressing specifically the larger spatial scale. Current assessment for aquatic systems focusses on the edge-of-field scale. Landscape level assessment should be more than an upscaled local assessment: not only accounting for a large number of spatially distributed edge-of-field situations, but also address the essential ecological aspects (and complexity) of spatially structured populations. For aquatic species this means explicitly accounting for spatial structure of habitat in e.g., an entire catchment, where some parts may be more exposed than others, account for the role of spatial processes (movement, dispersal, migration) providing functional connectivity and thereby playing an important role in e.g., local recovery, and accounting for larger temporal scale associated with spatial processes including local extinction and recolonization. A new endpoint in landscape level assessment may be the conservation of the spatial distribution (spatial integrity) of a target species population. Therefore, we explore a number of spatially implicit and -explicit endpoints for spatially structured populations, taking inspiration from e.g., metapopulation theory and the concept of source-sink dynamics. For an entire catchment, over a long period (>20 years) we apply a state-of-the-art individual-based population model for *Asellus aquaticus*, with individual sub-models for pesticide impact (reduced GUTS model for TKTD processes affecting survival and DEB - dynamic energy budget - for individual growth and development). The study is part of an extensive case-study for the Rummen catchment.

2.04.P-Tu052 Development of Sampling Methods to Facilitate Community Level Assessment of Chemical Impacts in Mesocosm Experiments for the Use in Aquatic System Models

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Mesocosm experiments are well established and regularly used as higher-tier test systems in environmental risk assessment and the authorization of chemicals. Due to their complexity, it is possible to assess the effects of stressors on multiple phylogenetic and zoological taxa from different trophic levels of aquatic communities. Study designs, endpoints and sampling techniques of mesocosm studies are mainly driven by e.g. the type of test item and its exposure, but also by requirements of general guidance documents and regulatory acceptance. For sampling methods in particular, this results in a focus on reproducible samplings over a relatively long period, with an emphasis on capturing a consistently high number of taxa and individuals for statistical evaluation. Therefore, results of these methods are well suited to follow and compare populations of each species over time and thus identifying direct and indirect effects. In recent years, modelling approaches in ecotoxicology have been developed and applied in the context of environmental risk assessment. Considerable efforts are currently being made to gain a deeper mechanistic understanding of the processes and effects of chemicals that lead to observed patterns at the community level. In a comprehensive ring study, control data from seven mesocosm studies are used connected with four aquatic system models (ASM) with the intent of evaluating their potential to explain observed effects on population dynamics and to inform future risk assessment. Arising from this ring study is the need for the development of new sampling approaches and techniques to produce representative mesocosm system level information suitable for model input and validation. Mesocosm studies are currently aimed to sample sufficient numbers of organisms for statistical analysis, and area- or volume-based quantification has not been a focus of method development. In addition, enrichment methods designed for comparison of treatment and control make it difficult to estimate the abundance of organisms for the entire system. In this study, we present a series of sampling approaches in untreated mesocosms to evaluate extrapolation factors and variances of different traps for common endpoints. This serves as a necessary link between information from mesocosm studies for regulatory purposes and modelling approaches, opening the opportunity to use huge amounts of available high-quality data for the refinement of ASMs to assess potential effects of chemicals or exposure patterns.

2.04.P-Tu053 Identification of Structural and Functional Endpoints in Fluvial Biofilms for Ecosystem-Service Approaches in Ecotoxicology

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Rivers provide a wide range of benefits for human populations while also being one of the most impacted ecosystems worldwide, particularly regarding chemical pollution. Within rivers, microbial biofilms are especially connected to the capacity of the river to provide supporting environmental services, including nutrient and carbon cycling, and pollutant mitigation. As part as the PRORISK-ITN, the aim of this work is to improve Environmental Risk Assessment (ERA) by incorporating functional and structural endpoints in fluvial biofilms as early warning signs of ecosystem service loss and ecosystem damage. To do so, we installed simplified fluvial systems to study the effects of benzotriazole-1H (a commonly used anticorrosive) in fish (*Gambusia*

holbrooki) with and without the presence of epilithic biofilms; in the composition and viability of the biofilms themselves (*i.e.*, algal and bacterial biomass, diversity, photosynthetic activity), and their potential as ecosystem service providers (ESPs) (*i.e.*, nutrient uptake, carbon cycling, toxicant removal). This experiment will facilitate the translation of chemical pollution effects in wild fish populations and ecosystem services associated with biofilms.

2.04.P-Tu054 Characterization of Biofilms From Microbial Fuel Cells With Raman- and Scanning Electron Microscopy-Based Techniques

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Microbial fuel cells (MFC) are an innovative approach in wastewater treatment aiming to reduce the pollution of greenhouse gases, to minimize the energy costs for wastewater treatment by generating bioelectricity and, consequently, to enhance the self-sufficiency of small- and middle-sized enterprises [1]. The process of electricity production of such exoelectrogenic biofilms is studied in many scientific fields, e.g., electrochemistry, biology, and biochemistry. Different strategies have been recognized of how a microbial consortium can transfer electrons to an electrode surface. One of the mechanisms can be the direct transfer of the electrons through outer membrane proteins or proteins that are aligned to a pilus. These proteins mainly contain heme-groups for conducting redox-processes. Another possible way is to transfer electrons via mediators such as humic substances, flavin molecules or carotenoids that can be easily oxidized and reduced. Electrons can also be transferred via interspecies contact from one cell to another as an electron transport chain to a conductive surface [2]. This work focuses on the characterization of microbial biofilms with imaging methods such as scanning electron microscopy (SEM) and Raman microspectroscopy (RM) to get a better insight into the complex structure of a biofilm that is used in MFCs for the anaerobic treatment of brewery waste. This includes the visualization of the allocation of inorganic compounds, as well as cellular components such as proteins, lipids, and polysaccharides and their characterization. SEM provides information on the three-dimensional structure of biofilms and their heterogeneity to complement findings obtained by three-dimensional Raman imaging. Both techniques help to shed a light on the underlying mechanisms of electricity production. [1] Gude, V. G.: "Wastewater treatment in microbial fuel cells – an overview", *Journal of Cleaner Production* 122, p. 287–307, 2016. [2] Semenc, L., Aracic, S., Mathews, E. R., Franks, A. E.: "Electron transfer between bacteria and electrodes", *Functional Electrodes for Enzymatic and Microbial Electrochemical Systems*, p. 93–170, 2017.

2.04.P-Tu055 Effect of Emerging EDCs on Crustacean Amphipod Behaviour

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Environmental pollution is still one of the biggest man-made threats to freshwater and marine environments. Of particular concern are emerging contaminants, anthropogenic chemical pollutants that have recently been detected in aquatic ecosystems but have not been assessed or regulated sufficiently. Effects of emerging pollutants on marine life are often unpredictable. Many of these compounds are targeting the endocrine system of an organism (endocrine disrupting chemicals, EDCs), and they impact not only survival but also behaviour, reproduction and development. Risk assessments of these emerging EDCs are difficult, given the lack of appropriate standardised tests and types of species tested. Here, we use the marine amphipod, *Echinogammarus marinus*, as a non-model species to assess the impact of three emerging EDCs on behaviour, measured in distance travelled over a time period after different light stimuli (alternating 2 min light off, light on) in crystallising dishes. Our results showed that dexmedetomidine, the active ingredient of novel antifouling paints for boats and a proposed EDC for octopamine receptors in crustaceans, significantly increased locomotion in amphipods after 4 days exposure (1-100 µg/L), while long term exposure up to 2 weeks suspended this effect. Triphenyl phosphate, a modern plasticiser and flame retardant used extensively around the world, did not affect distance travelled during the first few days of exposure, but significantly reduced locomotion in all concentrations (0.1 µg/L – 1 mg/L) after 2 weeks. Thiram, an agricultural fungicide, also used in personal care products and antifouling paint, had a significant effect on mortality with an LC₅₀ of 562 µg/L after 7 days. Effects on locomotion were clearly observed after 4 days with a significant decrease in distance travelled at higher concentrations (10-500 µg/L) and a further decrease after 2 weeks even in low concentrations (1-500 µg/L). While it is difficult to predict the ecological impact of those exposures on amphipods, an altered locomotive behaviour often comes with severe costs, e.g., in terms of ability of predator avoidance or energy consumption. Nevertheless, our results provide crucial information for future regulatory assessment of these commercially used compounds by authorities, while also promoting crustacean behavioural tests as part of the risk assessments process in the future.

2.04.P-Tu056 Crustacean Behaviour As an Assessment Tool for Emerging Environmental Pollutants

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With environmental pollution still a present threat to our marine environments, accurate and rigorous assessments of emerging chemicals are crucial to protect aquatic ecosystems. The marine amphipod, *Echinogammarus marinus*, and the common shore crab, *Carcinus maenas*, are key ecological species along marine coastal shores. Marine amphipods are top mesograzers, feeding mostly on macroalgae and reducing algal biomass, while also providing a food source for various predatory invertebrates, fish and birds. Although being essential to their ecosystem, amphipods are not commonly used as model organism for risk assessment of environmental pollutants. The common shore crab is more frequently used, but research is often focused on diseases and genetics. However, assessments of the effects of potential contaminants on the behaviour of invertebrates are rare due to insufficient information available for standardised protocols, suitable endpoints and controls. Here, we present information about crustacean behavioural assays using the marine amphipod and shore crab as model species to assess their behaviour using different endpoints

(distance travelled, activity) and behaviour stimuli (light, vibrations). The Zantiks behavioural measurement system was used to quantify and automate the crustacean's behaviour by tracking each animal in a crystallising dish and record their movement and activity. Many environmental pollutants, in particular pharmaceuticals, ingredients of personal care products as well as additives in plastics are known to act on the endocrine system of non-target species by mimicking or interfering with endogenous hormone functions or production. To make adequate assessment of future potential endocrine disrupting chemicals, appropriate positive controls are necessary, but are currently not well-established. In an attempt to provide such controls, we tested a variety of neurotransmitter and neurohormones such as serotonin, dopamine and octopamine on our crustacean models to assess their potential as controls alongside exposure experiments. Our results will provide better understanding of crustacean behaviour assays as well as promote their usage in assessments of environmental pollutants.

2.04.P-Tu057 Behavioural and Reproductive Effect of Plastic Additives on a Marine Amphipod

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Plastic additives are known for their endocrine disrupting properties even at low concentrations, which are suspected of causing behaviour and reproduction alterations in humans and animals. This study aimed to evaluate the impact of di-ethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP) on locomotive activity and sperm count of a coastal marine amphipod *Echinogammarus marinus*. The second aim was to compare intraspecies variability from the behavioural data. Amphipods were exposed to five concentrations (0, 0.5, 5, 50 and 500) µg/l of DEHP and DBP for 14 days. Behavioural analysis using an 8-min alternating dark and light protocol was performed on day 7 and day 14. Sperm count was analysed at the end of the 14 days exposure. DEHP exposure at all tested concentrations did not significantly affect the mean distance moved at the end of the experiment. DBP however resulted in a decline in the mean distance moved on day 14. The variability around the mean analysis suggests that animals exposed to DBP were more likely to react to a stimulus (light) during the 8 mins tracking time while DEHP had no significant variations. DEHP did not affect sperm counts; however, DBP significantly reduced sperm counts by 41.7% and 58.3% in 50 and 500 µg/l respectively. In conclusion, DBP impacted the locomotion of amphipods but not the same for DEHP, and DBP significantly reduced amphipods sperm counts. The variations in a behavioural dataset can potentially provide new information that might be lost or hidden. This has implications for marine ecologies and underscores the continued necessity for continuous review of plastic additives to combat negative effects transferable to the population levels.

2.04.P-Tu058 Exposure of *Mytilus Trossulus* to Oxytetracycline: Immunological Effects and Histological Alterations

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Potentially harmful or harmful compounds including pharmaceuticals are commonly found in marine waters and sediments. Among those, antibiotics and their metabolites are detected worldwide in relatively high concentrations, posing a risk to non-target free living species. In the current presentation, based on obtained experimental results, we encounter the hypothesis that oxytetracycline (OTC) exposure induces oxidative stress in marine invertebrates, activates cellular detoxification processes including Phase I and Phase II xenobiotic biotransformation enzymes and multixenobiotic resistance pumps (Phase III). Performed exposure experiment in which a model *Mytilus trossulus* was exposed to 100 µg/L of OTC resulted in no traces of cellular markers of the oxidative stress and no changes in expression of genes involved in detoxification processes (NADPH-CYP450 oxidoreductase CYP1L1, glutathione transferase GST?3, P-glycoprotein). Our results highlighted increased activity of phenoloxidase, a tissue-dependent activation of major vault protein (MVP) gene expression and a decrease in the nuclear factor kappa B-1 (NF-?B) gene expression in OTC exposed mussels. OTC-related endocrine disrupting effect was only confirmed at the gonadal tissue level (atresia of female gonads), but not on the aromatization efficiency in mussels. We therefore believe that the above-mentioned results together with an elevated number of regressive changes and inflammatory responses in tissues such as gills, digestive system and mantle (gonads) underline OTC effects on bivalves immunocompetence and general health, yet without markers of oxidative stress being detectable.

2.04.P-Tu059 Does Norfluoxetine Impact the Blue Mussel *Mytilus Trossulus* at Early Developmental Stages?

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In the last decade pharmaceutical pollution, due to their ubiquitous character and potential persistence, became an emerging concern in aquatic environment. It is acknowledged that pharmaceuticals entering freshwater and marine environments are derived from human activities based on daily usage of prescribed medication and their main source are treated water disposal from various sewage treatment plants. While environmental concentrations of various pharmaceutical compounds and their derivatives are continuously being detected in the water column, sediments and biota, their mode of action on non-target organisms are still insufficiently explored. Also, studies addressing the effects of pharmaceutical metabolites on aquatic organisms are still scarce. Norfluoxetine is an active metabolite of fluoxetine, a common antidepressant belonging to the group of drugs called selective serotonin reuptake inhibitors (SSRIs) used worldwide. Its presence has been found in many freshwater and marine environments adjacent to highly urbanized and densely populated areas. Particularly limited is the knowledge on the effects of SSRIs on early life stages of invertebrate benthic animals which are usually more sensitive to environmental stress. Thus, the aim of the present study was to elucidate potential effects of norfluoxetine on the early embryos and larvae of the model Mytilidae species inhabiting coastal areas of the Gulf of Gdańsk (the Baltic Sea). Early embryos and gametes of the blue mussel *Mytilus trossulus* were exposed to environmentally relevant concentrations of norfluoxetine (100 ng/L) in laboratory conditions. Fertilization success,

embryo development rates and morphological features of trochophore larvae treated with this compound were investigated against control conditions to better understand the effect of norfluooxetine on early life stages of these marine ecosystem engineers. The preliminary results showed significant effects of norfluooxetine on the bivalve *M. trossulus* embryos during first 24 hours post exposure pronounced in delayed development of early pre-trochophore stages. We therefore believe that the presence of pharmaceutical compounds of that type in the natural aquatic environments may have repercussions on embryo development, larvae growth and morphology, and eventually fitness of this ecologically and economically important species.

2.04.P-Tu060 Acute and Mechanism-Specific Toxicity of Coastal WATER Extracts From Swedish Industrial Cluster in Zebrafish and Marine Medaka Embryos

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Estuaries and coastal aquatic environments are daily threatened by the input of high levels of anthropogenic contaminants such as pesticides, industrial chemicals and pharmaceuticals. For a concrete risk management, it is of major relevance to examine the impact of complex chemical pollution within representative case studies. In this context the present study investigates the adverse effects of coastal water samples from Stenungsund and nearby freshwater streams in Western Sweden. Stenungsund hosts the largest petrochemical hub in Scandinavia and a middle city, which are expected to contribute into the pollution load and therefore play a role affecting aquatic organisms. This work had the overall aim to quantify adverse effects on marine and freshwater fish model species *Oryzias melastigma* (medaka) and *Danio rerio* (zebrafish) using multi-level endpoints on acute and mechanism-specific toxicity. Particularly, our study contributes to identify the ecotoxicological drivers of toxicity in this well-studied region, highly impacted by chemical industry, and will support the practical applicability of *O. melastigma* as a standard model species in marine ecotoxicology. To this end, fish embryo acute toxicity (FET) tests were performed at marine (*O. melastigma*) and freshwater (*D. rerio*) conditions. Embryos were exposed to large volume solid phase extraction (LVSPE) extracts in MeOH (marine: relative enrichment factor (REF) 5–80, 0.2 % solvent; freshwater: REF 5–40, 0.1 % solvent) shortly after fertilization. Embryos development was monitored every 24 h up to 192 (medaka) and 120 (zebrafish) hours post fertilization according to OECD 236. To investigate mechanism-specific endpoints on neuro- and cardiotoxicity with enzymatic biomarkers (EROD, AChE, CAT) and motor behavior alterations (spontaneous tail coiling, light dark transition test), embryos were exposed to sublethal concentrations of the extracts (EC5-20). Preliminary results with zebrafish indicated concentration-related increases of (sub)lethal effects and mortality for freshwater samples. In contrast, none of the marine water samples showed a distinct concentration-related increase in sublethal and/or lethal effects. In both species, most frequent effects were the impairment of the cardiovascular system as well as a reduced pigmentation. For a comprehensive interpretation of the current findings, the biological effect data will be compared with findings from chemical analysis which is currently in progress.

2.04.P-Tu061 Effect of Pesticide Prochloraz on Embryo-Larval Development of Common Carp (*Cyprinus carpio*)

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In the few past decades, water pollution caused by pesticides has become a commonly discussed issue including many scientific studies published on the presence of pesticide residues in the environment and their negative effect on non-target species. Pesticides are commonly used in agriculture and in households. Since they are persistent and might have bioaccumulation potential, they have been detected in surface water. Their residues can have a negative effect on aquatic biota, such as fish and other non-target organisms. Prochloraz is widely used imidazole fungicide, among other toxic properties it is also considered a potential endocrine disruptor. The aim of this research study was to describe the effects fungicide prochloraz on development of common carp early life stages using the Early-live Stage Toxicity Test according to the Guideline for Test No. 210. Five different concentrations of prochloraz (0.1 µg/L, 1 µg/L, 10 µg/L, 100 µg/L and 1 000 µg/L) were applied to water for 31 days with fresh solution replacements twice a day. In the study, statistically significant changes were observed at high tested concentrations. The results of the study showed that fungicide prochloraz had a negative effect on fish development at some tested concentration. This research was supported by project PROFISH – Mendel University in Brno, CZ.02.1.01/0.0/0.0/16_019/0000869 and IGA VETUNI 223/2021/FVHE.

2.04.P-Tu062 Assessing Critical Windows of Insecticide Exposure During Zebrafish Development

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During its development, the nervous system is most vulnerable to chemical exposure. Particularly, neuroactive chemicals, designed to target the nervous system, can interfere with tightly orchestrated developmental processes. Any disturbance of these processes can lead to impaired neuronal functioning, which severely reduces the fitness of the organism. Among others, insecticides represent a large group of neuroactive chemicals, which are inevitably released into the environment. Insecticides are applied primarily during spring and early summer such that insecticide application coincides with spawning season of many aquatic species, including fish. It is therefore unavoidable that fish and other species are exposed during early development. This study aimed to investigate which stages of the early development of zebrafish were most susceptible to sub-lethal insecticide

exposure. To cover different periods of neuronal development, zebrafish were exposed to two organophosphate (OP), two carbamate (CBM), and two neonicotinoid (NIC) insecticides in eight exposure scenarios. The latter included exposure from 0-1 days post fertilization (dpf), 1-2 dpf, 2-3 dpf, 3-4 dpf, 4-5 dpf, 5-6 dpf, 0-6 dpf, and unexposed control. After each exposure, except for the continuous, larvae were transferred to chemical-free medium and were raised until 6 dpf. Effects on the nervous system were assessed based on three behavioural assays. At 1 dpf, spontaneous movements of embryos within the chorion were measured with an automated tracking device. At 3 dpf, the touch-evoked response was tested by touching the tail of hatched larvae with a needle and measuring the distance moved. From 4 to 6 dpf, the locomotion in response to changing light conditions was assessed using the light-dark transition assay. Briefly, the OP dimethoate and the CBM pirimicarb significantly increased the number of spontaneous movements, while for thiacloprid (NIC) a significant reduction was observed. In the touch-evoked response assay, almost all insecticides, except imidacloprid (NIC), induced a reduction in distance moved. However, this effect predominantly occurred immediately after exposure, while larvae seem to have recovered from earlier exposure. Similarly, the locomotion at 4 to 6 dpf only was reduced when larvae were exposed shortly before. Although none of the exposure windows seemed to be critical when looking at locomotion, there is a tendency that earlier exposure leads to longer lasting effects.

2.04.P-Tu063 Implementation of Novel Methods in Ecotoxicology Assessment of Nanoparticle Toxicity to Aquatic Model Organisms (*Chironomus riparius*)

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Excessive production of nanoparticles has led to their accumulation in environment. Studies have shown that the current concentrations of nanoparticles in aquatic environment are only sublethal. For this reason, developing novel biomarkers that can detect negative effects of nanoparticles even at very low concentrations is very important. This way, appropriate remedial actions could be taken on time to prevent severe damage to aquatic ecosystems. Geometric morphometrics has a great potential to be used in ecotoxicology assessment, because of its ability to detect changes in fine structures on organisms and quantify them for statistical analyses. Tissue architecture alterations are very important indicators of toxic effect of pollutants. Histopathology analyses can present tissue morphology changes qualitatively as well as quantitatively. It is shown that nanoparticles could induce oxidative stress by generating ROS and comet assay is a simple method for detecting level of DNA damage that it causes. We have shown that sublethal concentrations of TiO₂, CeO₂ and Fe₃O₄ nanoparticles induce changes in *Chironomus riparius*. Using geometric morphometrics, alterations in mouth structures and wings in treatment with all three metal oxide nanoparticles was observed. Changes were noticed at a tissue level as well. Histology analysis of *C. riparius* larvae revealed vacuolization of digestive cells and Malpighian tubule epithelial cells in groups treated with nano-CeO₂ and nano-Fe₃O₄, as well as morphology changes of digestive system epithelial layer and fat body tissue in group treated with nano-TiO₂. DNA damage was observed by comet assay in groups treated with CeO₂ and Fe₃O₄ nanoparticles, where the severity of damage was concentration and particle size dependent. Geometric morphometrics and histopathology analyses revealed the structures that were the most sensitive to the presence of pollutants and could serve as potential biomarkers of nanoparticle toxicity. Harmful effect of nanoparticles on DNA molecule has indicated the importance of the comet assay method usage in ecotoxicology assessment. Our study showed that sublethal concentration of nanoparticles could have detrimental effect on aquatic environment and defining biomarkers of their toxicity is a very important step in taking actions to reduce risk of nanoparticle exposure.

2.04.P-Tu064 Assessment of the Effects of Cadmium, Samarium and Gadolinium on the Blue Mussel (*Mytilus edulis*): A Biochemical and Lipidomic Approach

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Rare earth elements are a group of 17 metallic elements. Their electronic configuration gives them particular properties which make their use in the composition of many products of our daily life essential. Their worldwide popularity disrupts natural biogeochemical cycles and enrichments in lanthanum (La), gadolinium (Gd) and samarium (Sm) are already observed in rivers. This study was carried out to assess the bioaccumulation and potential effects of 2 rare earth elements, Gd and Sm, in comparison with cadmium (Cd), on a marine sentinel species: the blue mussel (*Mytilus edulis*), combining biochemical marker analyses and a global lipidomic approach. Laboratory exposures were carried out at low doses of 50 and 500 µg/L in 13-L tanks of artificial seawater. Three tanks per element – Cd, Sm, Gd – each containing 13 mussels, were maintained in parallel with 3 other tanks without contamination. At the end of an 8-day exposure, during which the water and contamination were renewed every 2 days, the mussels were dissected, and a battery of biomarkers were performed on digestive gland, gills and mantle. Superoxide dismutase (SOD), catalase (CAT), and glutathion S-transferase (GST) activities served to monitor the oxidative stress. The neurotoxicity was analysed through the acetylcholinesterase activity (AChE). Acid Phosphatase (AcP) measures were used as a biomarker for immunotoxicity in haemolymph. Moreover, lipidomic analyses were performed using flow injection analysis coupled to high resolution mass spectrometry (FIA-HRMS) on lipid extracts from the digestive glands of mussels. Finally, the metal bioaccumulation was measured on exposed organisms by ICP-MS. All the metals were bioaccumulated by the mussels. Concerning the effects, Sm led to oxidative stress – induction of GST – in both gills and digestive glands. No significant effect was observed on the other biomarkers, *i.e.* phosphatase acid, CAT and AChE. Concerning lipidomics, no significant unsupervised discrimination between the conditions of exposure was highlighted by PCA. But, the exposures to Cd, Sm and Gd led to a total of 31 compounds up- or downregulated compared to the controls in the supervised analysis, using PLS-DA. Among them, one

compound was upregulated by the mussels exposed to the 3 metals and it would correspond to PIP3, which is involved in cell growth and survival. To conclude, biochemical and lipidomic approaches are complementary tools in ERA.

2.04.P-Tu065 Integrative Ecotoxicological Assessment of Rare Earth Element Effects on a Model Aquatic Community

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In the last decades, the increasing use of Rare Earth Elements (REE) in cutting-edge (smartphone, computer...) and green technologies (wind turbine, solar panel...) led to the release of anthropogenic REE into the environment, and especially in aquatic systems. However, still little is known about the potential effects of these emerging contaminants in the aquatic ecosystems. Therefore, we investigated REE aquatic fate and toxicity in indoor mesocosm exposure systems. A model food web was established with primary producers (macrophyte and microalgae), primary consumers (bivalve and crustacean) and a secondary consumer (fish). The organisms were exposed during four weeks to a mixture of REE (neodymium, gadolinium and ytterbium) at environmental concentrations. An integrative approach was implemented studying the speciation, bioavailability, bioaccumulation and toxicity of REE at the sub-individual and individual levels in a community context. This poster focuses on the effects on the bivalve *Corbicula fluminea*, a key filter-feeding benthic invertebrate of freshwater aquatic systems. A battery of biomarkers was measured by automated spectrophotometer in order to examine oxidative stress (glutathione peroxidase, superoxide dismutase, total antioxidant capacity), detoxification (glutathione S-transferase, acid phosphatase activities), cellular damages (lipid peroxidation, caspase-3 activity), energy metabolism (electron transport system, lactate dehydrogenase activities) and reserves (triglyceride, cholesterol, protein content) in the digestive gland and the gills of the bivalves. The results show a higher response of the biomarkers in the digestive gland with a significant increase of antioxidant and detoxification activities in the exposed bivalves relatively to controls. Moreover, an increase of lipid peroxidation was observed at the highest concentration. This work demonstrates that REE, at environmental concentrations, can induce toxic and oxidative stress and even cellular damages towards this key species in freshwater ecosystems.

2.04.P-Tu066 Ecotoxicological Effects of Closed Loop Scrubber WATER on Stickleback and Blue Mussel

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The shipping industry is responsible for the release of numerous contaminants, including sulphur oxides and polyaromatic hydrocarbons (PAHs), to the air and the marine environment. To reduce airborne pollution, a global 0.5 % sulphur limit for marine fuels was implemented in January 2020. The new lower sulphur limits can be reached via two different methods, by the use of low sulphur fuel or by installing an Exhaust Gas Cleaning System (scrubber). Today a large number of ocean-going ships have installed scrubbers to avoid using more costly low sulphur fuels. In a scrubber, the exhausts are led through a fine spray of water thereby “washing“ out particles and pollutants. There are different types of scrubbers, where the closed loop type recirculates the water to minimize discharge to the sea. Despite the name, a closed loop scrubber typically discharges 3-5 m³/h of polluted water. To study the effects of scrubber water, sticklebacks (*Gasterosteus aculeatus*) and blue mussels (*Mytilus edulis*) were exposed to water from a closed loop scrubber, in controlled laboratory experiments. Fish were exposed to 1% and 10% of the scrubber water for 4 and 14 days in a semi-static system, while the mussels were exposed to the same concentrations for 14 days. A battery of hepatic biomarkers on gene and protein levels were analysed in the fish while lysosomal membrane stability and byssus thread formation were analysed in the mussels. In addition, micronucleus analyses of fish blood cells were performed. Chemical analysis of the scrubber water showed the presence of several PAHs and metals. Results in fish show an induction of genes involved in detoxification (CYP1a), metals homeostasis (metallothionein), and oxidative stress (glutathione reductase, superoxide dismutase, glutathione S-transferase). Increased EROD activity and antioxidant enzyme activities show that upregulated gene expression led to increased enzyme function. Identification of 4-ringed and 5/6-ringed PAH in the bile samples of the fish confirmed the uptake of PAHs. However, no DNA damages were detected using micronucleus analysis. In mussels, no effect on lysosomal membrane stability was detected but it was clear that animals exposed to high levels of scrubber water had problems anchoring to the surface with byssus threads. Overall, results confirm that scrubber water carries pollutants capable of causing adverse effects in marine organisms.

2.04.P-Tu067 Effect Profile Characterisation of Complex Chemical Mixtures in a Wwtp-Affected Stream Using Effect-Based Methods

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Contemporary water pollution patterns are characterised by a steadily growing number of contaminants that are present in complex mixtures. The ecological consequences of these mixtures are often difficult or impossible to assess. Thus, it is important to conduct effect-based *in situ* studies – considering realistic scenarios – in biological systems to analyse the ecologically relevant effects of the combined exposure to pollutant mixtures. The present study, as part of a large case study within a well-studied low mountain stream in Central Germany (Holtemme River), is designed to (1) characterise the ecotoxicological potential along the

stream course, (2) identify hot spots of pollution and thus, (3) allow for an effect-based decision-making on suitable locations for subsequent experiments with caged fish. The case study aims to determine whether effect-based methods can be combined with established biomarkers, transcriptomics and genomics in feral fish to offer a diagnostic tool to assess ecologically relevant effects of anthropogenic stress in freshwater ecosystems. Water will be sampled, using Large-Volume Solid Phase Extraction (CHROMABOND HR-X) at 6 sampling sites along the Holtemme River. After enrichment, each sample will be analysed using a selected bioassay battery. The bioassays will comprise the Ames fluctuation test and micronucleus test to identify mutagenic and genotoxic potentials, respectively. Furthermore, ER-, AR- and Nrf2-CALUX assays will help to determine oestrogenic, androgenic and oxidative stress-related effects. The prolonged zebrafish embryotoxicity test (up to 120 hours post fertilisation, hpf) will be carried out to examine (sub)lethal effects and thus fish-specific toxicity. Moreover, locomotor behaviour of zebrafish larvae (96 hpf) at low sublethal concentrations will be analysed. These bioassays are complemented by targeted chemical analysis, using high-resolution LC-MS techniques to calculate toxic units and identify compounds strongly contributing to total toxicity. With this approach, a longitudinal characterisation of the ecotoxicological potential will be established and most importantly, hot spots of chemical pollution will be identified for the subsequent caging experiments. This study will help connect chemical pollution and ecological effects, fostering the understanding of causal links between environmental contamination and ecological impacts. This is considered a crucial input for future sustainable use of water resources.

2.04.P-Tu068 Multigenerational Exposure of *Daphnia magna* to Diazinon: Physiological and Molecular Responses

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Most of the ecotoxicological studies on daphnids are carried out on short to middle duration (48h/21 days). These classical tests are not adequate to evaluate the toxicity of environmentally relevant concentrations of chemicals. Indeed, organisms in surface waters are chronically exposed to pollutants, i.e. on several generations. These chronic exposures to low concentrations can induce subtle changes in health and physiology. Those can have negative ecological consequences on population levels and biodiversity. Life cycle exposures (continuous exposure from embryo to maturity) and multi-generational exposures (exposures extended beyond a life cycle) are therefore necessary to evaluate the ecotoxicological effects of compounds. In this study we aim in assessing sensitive makers such as physiological changes (reproduction, mortality and size) and biomarkers (acetylcholinesterase, Glutathione-S-Transferase and Cytochrome P450), over two generations exposed to diazinon, an insecticide regularly detected in surface waters. The concentrations range from 0.1 to 6 ng/L, which are realistic concentrations. In our study, we only exposed the first generation to diazinon (about 15 days) to mimic agricultural pollution, that is seasonal. The first results showed observable effects from 6ng/L, and this on the adults' size, the number of offspring and the egg laying time in the mothers exposed to diazinon. In the second generation, unexposed and therefore representing the consequences of parental exposure, only a slight trend was observed with increasing pesticide concentrations. The effects on biomarkers are currently under measurement.

2.04.P-Tu069 Assessing Anti-Androgenic ACTivity in Pond Water Using a Tropical Clawed Frog (*Silurana tropicalis*) Androgen Receptor Transactivation Assay

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Abstract – 300 words (2085 characters with spaces) Many amphibian populations are experiencing rapid global declines, with pollution being cited as a major cause. Of particular concern are pollutants that act as endocrine disrupting chemicals (EDCs). Laboratory exposure to various EDCs have been shown to induce a wide array of adverse reproductive effects in amphibians, particularly feminisation in males, including features of their morphology (e.g. secondary sexual characteristics) and physiology (e.g. changes in hormone levels and feminised gonads). These effects can occur through both oestrogenic and anti-androgenic activity of pollutants. Anti-androgenic activity is one of the most commonly detected endocrine disrupting activity recorded for environmental pollutants, however, very little is known on this activity in freshwater ponds where amphibians typically reside during breeding. Reports of anti-androgenicity – both for individual chemicals in the laboratory and derived from the testing of water samples – typically use transactivation assays which contain the human androgen receptor (AR). While there is a high sequence homology in the ligand binding domain between the human and amphibian AR (88 % or 82 % alignment between *Homo sapiens* and *Xenopus laevis* or *Rana catesbeiana*, respectively), the relative sensitivity of the amphibian AR compared to the human AR is not known. This is important in order to assess potential risk of anti-androgenic pollutants on the reproductive health of wild amphibians. With this purpose in mind, here we describe the development of a transactivation assay using tropical clawed frog (*Silurana Tropicalis*) AR transfected into immortalised liver cancer cells (HepG2). We also present preliminary data describing the activation of this receptor upon exposure to water extracts collected from common frog (*Rana temporaria*) breeding ponds in the UK. Results from this assay will be combined with sex determining gene expression data in common frog tadpole gonads to investigate for any relationships between anti-androgenic activity and the expression of selected target genes.

2.04.P-Tu070 Assessing the Impacts of Organic and Inorganic Contaminants on British Rivers: From Sediments to Otters

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A substantial proportion of British rivers are in poor ecological and chemical condition. Trace metal pollution has been a longstanding issue in the England and Wales, and recent reports also suggest that many British rivers are suffering from chronic

pesticide pollution. Both classes of pollutants have the potential to accumulate in the aquatic environment and in its inhabitants, and both can be highly toxic to aquatic life. Many rivers are still seeing catastrophic declines in freshwater fish, and chemical pollution is likely playing a role in this. However, there is a lack of knowledge of the general health status of fish populations, other than across population-level changes, and very few in-depth health assessments have been carried out in the UK. In addition, few pollutants are routinely monitored in sediments, and the exposure to biota is rarely assessed. To accurately evaluate and manage the risk to rivers, water and sediments pollution should be characterised in conjunction with effect-based monitoring methods. Of these methods, multibiomarker approaches across multiple levels of organisation have been developed to better characterise the physiological status of organisms. In this study, we have sampled water, sediments, invertebrates, and fish from across two contrasting agri-urban catchments in England. We will test our samples for a range of 31 trace elements and 64 current-use pesticides using ICP-MS and UPLC-MS/MS to map their distribution across the catchments and assess their bioaccumulation in the local aquatic fauna. The fish will undergo an in-depth health assessment using a series of biochemical, cellular, and histopathological assays. The gut microbiome of selected fish will also be sequenced using HiSeq to assess the implications of pollution on the microbiome and to examine the presence of antimicrobial resistance genes. In addition, and in collaboration with the Cardiff Otter Project, over 700 otter bones collected from four regions over two decades, including from two of the regions sampled in by our project, will be analysed for lead (Pb) accumulation, and potential Pb sources determined using stable isotope analyses. Concentrations will be mapped against background geology and anthropogenic sources, and a time-series analysis will determine whether bone Pb concentrations have decreased with reduced Pb emissions. Overall, this combined approach allows for a comprehensive ecotoxicological assessment of two typical British rivers.

2.04.P-Tu071 Effects of Leachates From Tyre Wear Particles on Marine Copepods

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Tyre wear particles (TWP) contribute a significant proportion to the microplastic pollution in the ocean, but the knowledge of their potential effects on marine organisms is still limited. This study aims to address some of this knowledge gap focusing on marine copepods, which are important organisms with a significant role in marine food webs and the biological carbon pump. The main objective of this project was to investigate the acute effects of TWP leachates on multiple zooplankton species including the cyclopoid *Oithona davisae*, the harpacticoid *Amonardia normanni* and the calanoids *Temora longicornis*, *Centropages hamatus* and *Acartia tonsa*. These copepod species differ in size, feeding mode and reproductive strategies. Acute toxicity experiments were carried out in the laboratory by exposing adult copepods of each species to a range of leachate concentrations (0.005 - 5 g L⁻¹) and using mortality (median lethal concentration, LC₅₀) as endpoint. All species exposed to the highest leachate concentration experienced 100% mortality within 72hrs, besides *A. normanni* (96.2%). Generally, the toxicity increased with increased leachate concentration and exposure time, therefore LC₅₀ values decreased over time for all species. Calanoids exhibited higher sensitivity, especially the small *A. tonsa* (728 ± 58 µm) and *T. longicornis* (672 ± 54 µm) which showed mostly 100% mortality in concentrations > 1.25 grL⁻¹, while the larger (853 ± 74 µm) *C. hamatus* exhibited higher tolerance (LC₅₀=1.5 grL⁻¹). The ambush feeder *O. davisae*, the smallest of the tested copepod species, was less affected than the larger calanoids (LC₅₀ for *O. davisae*=1.6 grL⁻¹). The harpacticoid copepod, *A. normanni*, showed a remarkable tolerance to TWP leachates (LC₅₀ = 3.2 grL⁻¹). Our results indicate that toxic effects of TWP leachates vary among different copepods taxonomic orders and that toxicity is most likely related to feeding/swimming strategies and species-specific defense mechanisms against pollutants.

2.04.P-Tu072 Impact of Tyre Wear Particle Leachates on Marine Phytoplankton

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Recent studies suggest that tyre wear particles (TWP) are abundant microplastics (MPs) in the environment. Once TWPs enter the aquatic systems, these particles leach out plastic additives that can be toxic to aquatic biota. However, little is known about the impact of TWP leachates on marine phytoplankton. The aim of this study was to determine the acute effect of leachates derived from TWP on three phytoplankton species: the cryptophyte *Rhodomonas salina*, the diatom *Thalassiosira weissflogii* and the dinoflagellate *Heterocapsa steinii*. Specifically, the effect concentration (EC50) for specific growth rate of each species was determined. Leachates were obtained by incubating < 250 mm TWP (1 g/L) in artificial seawater following a standard protocol. To be more environmentally realistic, we use a lower solid to liquid ratio compared to other standard protocols (50-100 g/L). Phytoplankton was exposed to leachates at different concentrations, and cell concentrations were measured every 24 h over 3 days. The dinoflagellate *H. steinii* was the most sensitive species to TWP leachates, with an EC50 of 23% leachate concentration after 72 h. *R. salina* and *T. weissflogii* exhibited EC50 values of 64% and 73%, respectively. These results indicate that leachates from TWP are toxic to marine phytoplankton. More field data is needed to evaluate the potential environmental impact of TWP leachates, particularly in coastal areas with close proximity to roads.

2.04.P-Tu073 Microplastics in Diet Affect Liver and Gills Function of Rainbow Trout (*Oncorhynchus mykiss*) Juveniles

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Plastic waste pollution is considered to be one of the most serious issues of our planet. Decades of increasing production and improper recycling caused that huge amounts of plastic waste enter the aquatic environment. Moreover, the widespread use of protective equipment due to Covid-19 pandemic, which is mainly made from various plastic materials, pollute the aquatic environment even more. In external environment, these plastic objects in large size may initiate creation of so-called microplastics (< 5 mm), which may be consumed by non-target organisms and could have negative effect on their health. The main task of this project was the evaluation of polystyrene (PS) microparticles effects on indicators of inflammation in rainbow trout (*Oncorhynchus mykiss*) juveniles. Tested fish were divided into four groups – with 0.5%, 2% and 5% concentration of PS microparticles in diet concurrently with the control group without the addition of microplastics in their feed pellets. Fish were fed with this diet for six weeks and the experiment ended with the blood and selected tissues sampling. In the highest tested PS concentration (5%), the inflammatory alterations were detected via changes in expression of selected cytokines in liver and gills and were confirmed by histopathological analysis of these tissues. In addition, the biochemical analysis found a decrease in ceruloplasmin activity and ammonia concentration in the same PS group. In conclusion, PS microparticles can affect health indices of *O. mykiss* and thus the potential risk for aquatic environment and even human consumption should be considered. Project was funded by the ERDF/ESF “PROFISH” [no. CZ.02.1.01/0.0/0.0/16_019/0000869].

2.04.P-Tu074 Variability of Tolerance to Methylisothiazolinone Among Independent Lines of *Daphnia pulex*

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Isothiazolinones are a family of broad-spectrum biocides widely used in industry and consumer products. These compounds are recognised as strong irritants, with the first cases of contact allergies being reported in 1984, resulting in their restriction by EU legislation. The less harmful of these molecules – methylisothiazolinone (MIT) – is still being used in cosmetics, household cleaners, hygiene products and various industrial applications. Despite this widespread use, the release of MIT from urban sources to aquatic environments and its potential impacts on aquatic organisms have received little attention. Few studies have reported the toxicity of MIT to non-target species. The present work addressed this current knowledge gap by evaluating acute MIT toxicity to *Daphnia pulex* (Cladocera), as well as its sublethal effects on fertility and growth under environmental concentrations. To account for intraspecific genetic variation in sensitivity, the design involved six clonal lines of *D. pulex* stemming from distinct natural populations or commercial strains. Clones exhibited strong variation in their responses, which points to the need to incorporate genetic diversity into ecological risk assessment procedures.

2.04.P-Tu075 Acute and Chronic Toxicity in the Cladoceran *Daphnia Curvirostris* Exposed to Inorganic Turbidity and Glyphosate

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Intensive agriculture is actually one of the most widely techniques to satisfy the food claims in the world, which among other things makes increasing use of herbicides like glyphosate. In countries like México, other type of agriculture is extensive, realized commonly in slopes and in sites with grades, promoting the loss of soil and dragging sediments to the catchment basins. Pesticides and inorganic particles are introduced to water bodies by runoffs causing increased inorganic turbidity and negative effects in aquatic organisms, specifically on the filtering zooplankton. This study evaluated the glyphosate toxic effect, in presence and absence of inorganic turbidity, on reproduction, survival and the biomolecules in the cladoceran *Daphnia curvirostris*. The acute toxicity (48 h) of glyphosate (FAENA[®]) was determined applied both individually and in combination with clay presence (0.1 mg/L). For the chronic bioassay (life cycle) were used the following sublethal concentrations: LC_{0.1}: 1.34 mg/L, LC₁: 1.95 mg/L, LC₅: 2.72 mg/L y LC₁₀: 3.25 mg/L, each one with 0.1 mg/L of clays, one control and a control with 0.1 mg/L of clays; these were feed with the microalgae *Pseudokirchneriella subcapitata* (800,000 cel). The calculated LC₅₀ for FAENA[®] was 4.92 mg/L without clays and 6.10 mg/L with clays. Chronic bioassay (FAENA[®] with clays) results showed that the LC₁₀ affected the survival of the 80% of the cladocera at the 32th day; both controls completed their life cycle at the day 43th; chronic bioassay (FAENA[®] without clays) results showed that the LC₁₀ and LC₅ affected the survival of the neonates the first few days. There was a significant reduction in the number of neonates and litters, mainly those exposed to the highest concentrations of glyphosate + 0.1 mg of clay. The highest percentage of abortions (total of neonates: 6%) was recorded in LC₁₀ with clay. According to macromolecules, adults of *D. curvirostris* had high protein content, followed by carbohydrates and lipids in all FAENA[®] with clays concentrations; lipids were significantly reduced in all treatments. In neonates were registered highest lipid contents, followed by proteins and carbohydrates. Results of this study indicated that the clays modify significantly the glyphosate toxicity, moreover, the exposure of *D. curvirostris* to glyphosate and clay caused damage to survival, reproductive and macromolecule content, specially the decrease of lipids, which are important for the reproduction.

2.05 Lab and field based experimental approaches to investigate ecosystem integrity under multiple stressors

2.05.T-01 The Effects of Stressor Dominance at Environmentally Relevant Levels on the Freshwater Gastropod, *Lymnaea stagnalis*"

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Human activity is driving biodiversity loss at an alarming rate and multiple interacting stressors are recognised as driving many of

these declines. However, due to the practical limitations associated with multifactorial experimental designs, a new approach to investigate the effects of environmental stressors is needed. Here we assess combined effects of three ubiquitous environmental stressors (pollutant mixture, invasive predator and global warming) utilising a novel testing paradigm based on the ‘dominance’ theory. The central tenet of this theory is that when one or more stressors are present at sufficiently high levels, this drives the observed effects. Under this scenario, the addition of co-stressors at lower levels will have little impact on the level of response. In order to test this theory, stressors were combined with each stressor at a ‘dominant – EC30’ level, either alone or in combination with additional stressors at ‘negligible – EC10’ levels and the selected levels encompassed environmentally relevant exposures. Effects on survival, growth, reproduction and behaviour in *Lymnaea stagnalis* upon long-term exposure (~ 5 months) were recorded. When stressors were applied singly at the ‘dominant’ EC30 exposure level, negative effects on reproduction (embryo viability) were observed in response to pollution, avoidance behaviour was observed in response to predator cue and stimulatory effects on reproduction (accelerated egg production) was observed in response to temperature. Supporting our hypothesis, there were no differences in these effects when compared with co-exposure with other stressors at negligible levels. However, additional effects were observed for all stressors under the multiple stressor exposure scenario when compared with their respective single EC30 exposure. Observed effects were similar between the different multiple stressor exposure scenarios, and therefore, the overall stressor levels appeared to have more importance than the individual stressor identities. This could be due to a general stress response leading to changes in energy budget allocations. Findings from this study will provide crucial information on how combinations of stressors interact to affect aquatic organisms, reflecting how human activities are contributing to biodiversity loss within natural systems.

2.05.T-02 Combined Effects of Droughts and Pesticides Cocktail on the Biomass and ACTivity of Aquatic Biofilms

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The potential adverse effects of mixing different pesticides (pesticide cocktail) in freshwater ecosystems subjected to extreme hydrological events such as droughts has been poorly explored. For this reason, there is an urgent need to increase our knowledge in this field to propose suitable pesticide mitigation strategies in the context of future global change scenarios affecting aquatic ecosystems. In this study, a microcosm experiment was performed to assess the effects of two types of drought events caused by hydropeaking (short and highly frequent drought episodes- HF_SD) and agricultural practices (long and low frequent drought episodes- LF_LD), with an immersed control (IC), on aquatic biofilm performance before and after the exposure to a cocktail of pesticides (composed by fungicides, insecticides, and herbicides). Different biomass and functional parameters were analysed in biofilm to determine the combined impact of droughts and pesticides. Results showed that lower bacterial densities in biofilms were measured after the cocktail application. In contrast, the proportion of live bacteria was higher after the application of the pesticides cocktail in the three hydrological conditions. Algal biomass measured as chlorophyll-*a* concentration was significantly lower under drought conditions comparing to the IC and these differences were more remarkable after the application of the cocktail of pesticides. Microbial respiration corrected by organic matter content in the biofilm was strongly lower in the LF_LD condition compared to the IC in the overall experiment ($F=25.972$, $p<0.0005$). Moreover, an interaction between droughts and pesticides ($F=2.738$, $p=0.043$) revealed that respiration rates increased in LF_LD and IC after the cocktail application. Besides, results from community-level physiological profile analyses revealed significant differences between drought treatments, especially in biofilms exposed to LF_LD with stronger contribution to polymers degradation ($F=24.211$, $p<0.0005$) causing a shift in biofilm heterotrophic behaviour. Fundamental knowledge to determine the effects of multiple stressors such as a combination of pesticides impact and droughts exposure on microbial biofilm communities will help to improve the management of freshwater ecosystems under the global change.

2.05.T-03 Dietary Effect Pathway in Gammarus fossarum (Crustacea; Amphipoda): Influence of Land-Use and Leaf Substrate

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Ecosystem functions provided by microbes in low-order streams, such as microbial colonization of allochthonous leaf litter that increases the leaf palatability for macroinvertebrate leaf-shedders, maybe globally be threatened by increasing chemical stress due to an intensified agricultural activity. The observed bottom-up directed effects on leaf-shredders may, however, depend on a previous stress exposure of microbes that shaped the microbial community and favored more or less palatable microbial species. To test this, we studied how the feeding rate and egestion of the leaf-shredder *Gammarus fossarum* (Crustacea; Amphipoda) are modulated when feeding on two types of leaf substrates (black alder, European beech, and a combination thereof) that were colonized by microbial communities with distinct exposure histories (pristine vs. vineyard runoff [VYRO]), using a fully-crossed 2x3-factorial test design over 21d (n=40). Additionally, we assessed the exoenzyme activity and community composition of the leaf-associated microbial communities (currently being performed). Gammarids fed slightly more (~10%) on alder conditioned in the pristine stream than on leaves conditioned in VYRO. The same pattern was observed for the combination of alder and beech. In the case of beech, the opposite trend was observed, namely gammarids fed more (~30%) on leaves conditioned in VYRO than at pristine sites. The gammarids’ feeding rate thus showed an effect of the substrate ($p=0.015$) and interaction of community history with the substrate ($p=0.005$). A similar pattern to the feeding rate was also observed for the egestion ratio, however, the effect was only significant for substrate ($p<0.05$). The higher consumption of beech conditioned in VYRO might be a mechanism of compensation due to microbial community shifts that reduced the nutritional value of the leaf litter and forced gammarids to consume more in order to meet their energetic requirements. Against this background, energy reserves will be analyzed to better

understand physiological consequences at the consumer level. A very distinct pattern of the enzyme activity was found on the differently conditioned leaves, with peroxidase showing a significant interaction between community history and substrate ($p=0.016$). Together with the pending data, we will discuss the consequences for gammarids, a keystone organism in stream ecosystems converting leaf material to particulate organic matter and serving as prey for higher trophic levels.

2.05.T-04 Metabolomics Insights in the Response and Tolerance of Stream Biofilms to Chemical Stress in Urban Context

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Recent evidences highlighted the effect of urban effluent on the structure and functions of downstream biofilms associated to increased tolerance to further chemical stress. Nevertheless, the underlying (molecular) mechanisms of these responses remain poorly understood while usual descriptors provide a partial picture of the phenotype of the communities under chemical stress. In this context, the present study aims to gain knowledge about the molecular/biochemical responses of stream biofilms under urban stress through the implementation of untargeted metabolomics approach. To do so, following exposure in indoor channels connected to a urban effluent, the metabolomic responses of the biofilms were characterized in parallel of structural and functional responses. First, no significant effects were noted on the photosynthesis, respiration, primary/secondary production, as well as on biomass whereas exposure to the unfiltered effluent led to tolerance acquisition to further chemical stress likely associated to significant shift in the microbial diversity. Then, the metabolomic profiles showed discrepancies between all the tested conditions demonstrating a clear effect of the effluent on the molecular phenotype of the biofilms. Moreover, the clear separation between unfiltered and ultrafiltered conditions highlighted the potential contribution of the microbes from the effluent in the response. Such pattern paralleled with microbial diversity one. The strong influence of the microbes was further confirmed through HCA showing that control and ultrafiltered conditions were clustered together. Further trends analysis combined with pathways analysis revealed potential impact on porphyrin and chlorophyll metabolism, among others. Finally, statistical comparison between the metabolomic profiles of raw vs ultrafiltered conditions highlighted that only 11% down- and 18% up-regulated signals contributed to the tolerance of the biofilm. Overall, this study demonstrates that the metabolomic response is more sensitive than usual descriptors through its ability to discriminate all the experimental conditions. Moreover, our results showed that microbes strongly contribute to these various molecular/biochemical responses, as well as to tolerance acquisition. Further investigations are ongoing to confirm the identity of the candidate metabolites and pathways and discriminate their link to urban microbes vs chemicals associated to the tolerance acquisition to chemical stress.

2.05.T-05 Interactive Effects of Metals and Carbon Nanotubes in a Microcosm Agrosystem

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Agricultural soils are exposed to multiple contaminants through the use of agrochemicals or sewage sludge, introducing in the environment metals and nanomaterials among others. Among nanomaterials, carbon nanotubes (CNTs) are known for their large specific surface area and adsorption capabilities, possibly modifying the behavior of other element. However, to date, very little is known about the impacts of such interactions in agrosystems. In this study, we aimed at understanding the transfer and toxicity of contaminants (Cd, Pb, Zn and CNTs) in microcosms configuration including native soil bacteria, earthworms and lettuce. After a 6 week exposure, no effect of the addition of CNTs to metal-contaminated soils was detected on bacterial concentration or earthworms growth. However, in plants, an interactive effect between CNTs and metals was evidenced: the addition of CNTs led to a biomass loss (-22%) together with a flavonoid concentration increase (+27%). In parallel, the addition of CNTs led to differential impacts on elemental uptake in lettuce leaves possibly related to soil matter content. For earthworms, the addition of CNTs resulted in an increased body elemental transfer in the soil with the highest organic matter content (Pb: + 34% and Zn: + 25%). The results of this experiment provide evidence of interactions between CNTs and metals and show the potential for synergistic and antagonistic interactions depending on the organism considered. Finally, the results of this study raise the issue for authorities to review the current model by which contaminant risk is currently assessed. It is necessary to include the impact of contaminant interactions (cocktail effects).

2.05 Lab and field based experimental approaches to investigate ecosystem integrity under multiple stress (Virtual Only)

2.05.V-01 Great Pond Snail (*Lymnaea stagnalis*) As Sentinel Species for Biomonitoring Anthropic Pressure Impact in Two Algerian West Rivers

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Most of the rivers in the North-West of Algeria have a catchment considerably influenced by localities and agricultural activities, which can generate discharges of various pollutants and weaken biodiversity. This study aims in assessing the risk of two rivers submitted to anthropic pressure: El Malah river (EM), and Sidi Djelloul river (SD), using a gastropod species *Lymnaea stagnalis* as a bioindicator. To reach this goal, we selected 09 sites along both rivers, and we analyzed the following parameters: 1) abundance of the population, 2) biomarkers activities (Acetylcholinesterase (AChE), Glutathion-s-Transferase (GST), Lipid Peroxidation (LPO)), 3) concentration/bioaccumulation of trace metals (Cd, Pb, Cu, Zn, Cr, Ni), in the sediment and specimens.

Sampling was done for four seasons during 2020/2021. The ecological distribution study demonstrated a longitudinal decrease in the abundance of the population along both rivers from upstream to downstream. Moreover, no specimen was recorded in the areas submitted to wastewaters discharge, to discontinuous drying out due to the lack of rain, or to a high irrigation rate. The results also showed a significant increase in GST activity and a significant decrease in AChE activity in the snail from SD river, suggesting greater exposure to insecticides at this site. Indeed, many insecticides are cholinesterase inhibitors. An increase in GST activity could indicate general oxidative stress. However, no statistically significant variation was observed in LPO activity. In site 2 of EM river, high concentrations of trace metals in sediment were recorded in fall coupled with high bioaccumulation in the specimens, especially for lead (Pb) 33.44 µg/g d.w., 14.94 µg/g d.w., respectively. Lead could originate from chemical fertilizers or wastewaters. This study represents a first step in the risk assessment of rivers highly influenced by anthropic pressure in Algeria. It allowed demonstrating that there is a high and chronic risk for invertebrate communities and the fluvial ecosystems in general. Measures must be implemented, such as urban wastewater treatment and the promotion of extensive agriculture.

2.05.V-02 Microscale Climate Exposure System for Assessing the Effect of CO₂, Temperature and UV on Microalgae
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Due to the intense anthropogenic activities and accelerated climate change, marine ecosystems are facing enormous challenges to survive the effects of rising temperatures, ocean acidification and changes in background Ultraviolet (UV) radiation. To better characterize the effects of changing environmental factors on marine ecosystems, a small-scale exposure system that can effectively simulate multiple climate change effects using high-throughput bioassays is desirable. In the presented study, a multiple stressor exposure system based on micro-climate units that can fit into traditional laboratory incubators was constructed to assess the effects of climate relevant stressors such as CO₂, temperature and UV radiation on small marine organisms. The performance of the exposure system was tested using a chronic toxicity test with the diatom *Skeletonema pseudocostatum* (NIVA BAC 1) exposed to varying levels of CO₂, different temperatures and UVB radiation for 72 hours. Changes in physical parameters, including pH, dissolved inorganic carbon (DIC), total alkalinity (TA), temperature and salinity in the medium, as well as several toxicity endpoints such as reduction in growth, photosynthetic efficiency and content of chlorophyll a were characterised. The results verify that the exposure system can maintain a stable CO₂, temperature and UVB irradiance for extended durations of time, suggesting that the system can successfully simulate changes in ocean acidification, global warming and UV exposure. Toxic responses measured in the bioassays were typically dose-dependent, which is comparable to other exposure systems, and suggested that the multiple-stressors exposure system has utility for assessing both effects and climate adaptation to single and multiple stressors. Acknowledgment – This project was financed by the Norwegian Ministry of the Environment through the Norwegian Institute for Water Research basic funding (SIS-program on Ocean Acidification), the Research Council of Norway project 223268: Centre of Excellence funding scheme, and NIVAs Computational Toxicology Program (www.niva.no/nctp).

2.05 Lab and field based experimental approaches to investigate ecosystem integrity under multiple stress (Poster)

2.05.P-We021 Aquatic Ecotoxicology Testing : Methodological Adaptation of Mesocosms for Complex Substances Like Paraffin Oils

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Paraffin oils, used as insecticide and acaricide for crop protection, can be challenging when they have to be tested for effects towards specific aquatic organisms in mesocosms systems : indeed, as for the same type of substance, their physico-chemical properties, hydrophobicity and very low solubility in water with a density less than 1 in particular, requires to use specific equipment and facilities. A customized experimental system was run in 2020 to assess the environmental impact of paraffin oils: open-stream outdoor fresh water mesocosm i.e. 16 channels artificial open streams rivers (max. 200 m³/h in total and 7,5 m³/h each) fed by Gave de Pau (southwest France) allowing to test different exposure conditions. The injections of the tested substance occurred through a shearing valve directly into the stream. The shearing valves generate an intimate emulsion which micro-disperses the formulated active substance in water and allows a homogenous dissolved concentration in the streams. Two concentrations have been tested in accordance with the Good Agricultural Practices, as well as a negative and positive control (pentahydrated Copper sulfate) control. Details of the design are presented in this poster, including the different monitored parameters. Results of the 28 days experiments are subject to another poster.

2.05.P-We022 Honey Bee Lifecycle Assessment and Homing Success in Field Observations With the Help of Visual Bee Monitoring Technology

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At the moment there are fast advances in the technology available for bee monitoring. There is a strong desire to enhance the knowledge creation on bee health and to get a better understanding of how available feed resources contribute to bee health. New technologies such as bee counters and other monitoring devices/equipment carry a vast potential to gain insights into these questions. They make it possible to move from assessments of individual time points to continuous observations. A new technology besides counters to observe survival, and/or behavior are RFID chips. The chipped bee's homing flight behavior can also be observed to find out if there is an influence of a plant protection product on the orientation of the bees (OECD GD 332).

To increase the amount of information on colonies it would be advantageous to include information about the life cycle of individual bees with bee counters. Field testing of plant protection products is already very labor-intensive. If homing behavior would be included in a field study with RFID chips additional hives would be needed. But hives use landscapes in different ways so effects observed on a hive equipped with RFID readers are not necessarily relevant for the other hives placed in the same landscape. Since apic.ai and EAS Ecotox are partners in the improvement of a visual bee monitoring technology in the research project OCELI (FKZ 281C307B19), a proof-of-concept experiment was performed. The apic.ai monitoring systems with computer vision technology were used to observe individual bees. Queen markers were attached to bees to identify them individually. This approach could enable the inclusion of life cycle, homing success, and individual behavior in studies where visual monitoring technology is already in use to assess other behavioral endpoints like activity, pollen collection or share of foragers. For the study, two colonies were marked in one interval of one week with a different color. At each marking, young and forager bees were marked. The aim of the experiment was to find out if the OCELI bee monitor could distinguish individual bees moving in and out of the hive. It was assessed whether there are colors that work better than others and how well the identification of the numbers printed on the plates would work. Furthermore, the success of the marking was determined for an experienced and inexperienced group to see how easy the methodology would be transferable. This work was supported by grants from the German BMLE.

2.05.P-We023 Trophic Dynamics of Mercury in the Baltic Archipelago Sea Food Web

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At the present day, mercury (Hg) exceeds its natural environmental concentrations with well-documented biomagnification and toxic consequences for biota. The present study, therefore, investigated trophic Hg dynamics in the benthic and pelagic food chains of the Baltic Archipelago Sea, known for its high pollution. While traditional biomagnification (BMFs) and trophic magnification factors (TMFs) were used, special attention was paid to the influence of ecological (food chain, trophic position) and ecophysiological traits (thermoregulatory capacity). The study species, composing of 3 seabird, 17 fish, 10 invertebrate and 4 primary producer species, were categorised based on their habitat (benthic, pelagic or benthopelagic) and thermoregulatory capacity (homeotherm, poikilotherm), and ¹⁵N-based trophic position (TP) calculations were carried out according to their habitat. Hg concentrations (mean \pm SD μ g g⁻¹ dry weight: seabirds 1.21 \pm 1.18; fish 0.20 \pm 0.278; invertebrates 0.023 \pm 0.0208; primary producers 0.029 \pm 0.071) increased significantly with TP in the species of both benthic and pelagic food chains, with all TMF exceeding one (benthic food chain [range] 3.0-3.2; pelagic food chain 4.4-5.6), indicating biomagnification. In the models excluding seabirds, TMFs were significantly higher in the pelagic food chain, possibly due different in species biology, such as metabolic differences and narrower niche width of pelagic species, causing more efficient transfer of Hg in the food chain. Most BMFs were >1, further demonstrating Hg biomagnification. There was higher variation in the BMFs when normalising for the TP difference between the prey and predator (mean \pm SD: 14.0 \pm 44.5, range: 0.1 – 508.4) than when not (mean \pm SD: 10.7 \pm 10.3, range: 0.05 – 82.2). The highest BMFs corrected for TP were not caused by a large difference in Hg concentrations between prey and predator, but rather by a small difference in TP, showing how a small difference in TP can artificially inflate the BMF. Currently, there is not one single waterproof BMF calculation and different formulas each present trade-offs between ecological reality and statistical applicability. Our results demonstrate Hg biomagnifying in both benthic and pelagic food chains of the Baltic Archipelago Sea. Trophic magnification of Hg seems to be more efficient in the pelagic food chain, highlighting the important role different ecological pathways can have in contaminant exposure of the species.

2.05.P-We024 Characterization of the Metabolomic Response of Freshwater Biofilms to Urban Wastewater Effluents

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Urban wastewater treatment plants (WWTPs) release large amounts of pollutants (e.g. chemicals, microbes) into aquatic environments that may impact exposed organisms. Among these organisms, aquatic biofilms, as a complex assemblage of microorganisms with a short life cycle (e.g. microalgae, bacteria, etc.) and by their key role in aquatic ecosystems (e.g. primary production), are relevant and increasingly used to investigate the impact of chemical contamination at the community level. Despite increasing knowledge on the impact of chemical stress on these communities, the underlying (molecular/biochemical) mechanisms remain poorly described while usual descriptors provide a partial picture of the phenotype. To tackle this issue, untargeted metabolomics approach is relevant through the simultaneous characterization of chemical exposure and the global response of the whole biofilm. In this context, this study focuses on the characterization of the potential impact of urban WWTPs on aquatic periphytic microbial communities by implementing an untargeted metabolomic approach. To do so, biofilm colonization/exposure was carried out during 4 weeks at upstream and downstream sites from three WWTPs along the main tributary of Arcachon Bay (Belin Béliet: upstream, Salles: intermediate; Mios: downstream). First, multivariate analyses (PCA and HCA) showed discrepancies in the metabolomics profile between the three WWTPs and between upstream and downstream site of each WWTP. This difference is more marked at the downstream site (Mios), likely because of increasing disturbances along the tributary (e.g. detection of pesticides only on the downstream site). Moreover, the strong discrimination between upstream and downstream sites at Mios suggested that this WWTP is a source of pollutants. At this site, further univariate analysis combined to pathways analysis allowed preliminary annotation (i.e. based only on MS1) that highlighted a potential impact on the respiration and photosynthesis pathways. Further investigations are ongoing to improve the annotation through the analysis of

MS² spectra and by using an internal database. Overall, this study confirms the relevance of untargeted metabolomics to highlight potential impact of urban discharges on aquatic microbial communities.

2.05.P-We025 Preservation of Environmental DNA (eDNA) in WATER Using Cationic Detergents

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Abstract: Environmental DNA (eDNA) techniques are gaining attention as cost-effective, non-invasive strategies for the detection of many types of animals, especially in aquatic ecosystems. These include common, invasive, indicator and endangered species in ponds, lakes, rivers, streams, and oceans. Filtration of water using glass fibre and nitrocellulose filter paper are commonly used to extract eDNA from the water and later used for the sequencing or quantification. As eDNA is more prone to degradation immediate filtration is usually needed and can be done by onsite filtration. However onsite filtration can be difficult in remote areas and sometimes the quality of water challenges filtration rate. To overcome these difficulties, we have analysed the efficacy of using different water preservation methods utilizing addition of cationic detergents. Four different cationic detergents, Benzalkonium chloride, Benzethonium chloride, Cetylpyridinium chloride and Cetyltrimethylammonium bromide have been tested for their eDNA preservation efficiency in water. A mesocosm study (5 days) was conducted using zebrafish (*Danio rerio*) as a test organism. The water was then treated with and without the addition of 0.01% cationic detergents. To test the efficiency of the additions the water was collected and filtered from 0 to 96 h after collection. The results showed that all cationic detergents were equally effective at preserving zebrafish eDNA in water while untreated samples showed substantial degradation. At present eDNA studies have adopted on-site filtration followed by filter fixation. Addition of cationic detergents can protect eDNA in water samples allowing for transport to the laboratory for extraction as they prevent the loss of eDNA in water samples. In the ongoing work with development of the eDNA method we will establish a ddPCR protocol to improve the sensitivity of the assay and validate the method using environmental lake water with and without addition of cationic detergents.

2.05.P-We026 Periphyton Communities Under Multiple Stress - Detecting Community Shifts Via Dna-Metabarcoding

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Assessing the effects of multiple stressors in surface waters is currently an important topic in ecotoxicology research. Multiple stress may include combinations of chemicals or excessive input of nutrients, hydromorphological degradation, temperature increase or salinization. Due to the increasing effect complexity associated with the occurrence of multiple stressors, e. g. as a result of global climate change, a better understanding of the interactive effects of stressors on communities and their responses is required. In freshwater systems, periphyton communities contribute to primary production, represent an important food source for grazing animals and host a large biodiversity including bacteria and algae. Thus, chemical stressors, such as antibiotics or herbicides, can directly interfere with these communities. Species turnover in the periphyton community composition (horizontal interaction) could in turn indirectly affect the food webs depending on primary production and grazers (vertical interaction). The aim of our study, therefore, was to investigate the combined effects of chemical stress and nutrient enrichment on the community composition of freshwater periphyton. Using artificial indoor streams as microcosms, we set up experiments by colonising natural periphyton on ceramic tiles and exposed them to four different concentrations of the antibiotic ciprofloxacin and the herbicide propyzamide (i.e., chemical stress; 0, 1, 10, 100 and 1000 µg/L), as well as nutrient poor or nutrient rich media (i.e., nutrient stress; modified Borgmann and KUHL, respectively). As periphyton communities underlie seasonal changes, we performed two experiments during winter and two during summer. Via DNA-Metabarcoding, we evaluated the species composition of microeukaryotes and prokaryotes within chronically exposed periphyton (14 days). The data is currently being analysed and the results will be the focus of this poster.

2.05.P-We027 Link Between Glyphosate Exposure and Teratological Forms in Freshwater Benthic Diatoms

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The Water Framework Directive stipulates that water bodies must achieve “good ecological status” in the EU. Although many indicators exist, they are not always able to reveal the subtle effects of low episodic pressure over long exposure times. It is therefore essential to develop early warning indicators of the surface water quality. For this purpose, the study of phototrophic biofilms seems particularly relevant because of their major role in aquatic ecosystems. The Cleurie river (Vosges, North-eastern France) constitutes an ideal study site for testing the sensitivity of indicators and their relevance in the WFD context. Due to industrial activities (textiles), this forested headwater stream is exposed to a complex cocktail of molecules characterized by relatively high concentrations of glyphosate (i.e close to environmental quality standards) and aminomethylphosphonic acid (AMPA), its main degradation product but also dyes and optical brighteners. A preliminary study on the watercourse indicates that teratological forms in the diatoms seem to be more abundant downstream of the discharges. The purpose of this study was to determine if the deformities of benthic diatoms would constitute an early and more sensitive marker than the traditional structural indices. In this context, our objective was to estimate *in situ* the ecotoxic potential of this complex contamination on the diatoms morphology and to identify if these effects can be related to the release of glyphosate and AMPA in the Cleurie river. For this

purpose, diatom community structure, presence of deformations and water physicochemistry were monitored on five sampling sites along the river, at seven occasions along the year. In parallel to this field approach, bioassays were performed in 96-well microplates to evaluate the toxicity of glyphosate and AMPA on diatoms under standardized conditions. Short-term (7 days) exposure of the model species *Nitzschia palea* was conducted to determine the concentrations of contaminants inhibiting 5, 10 and 50% of their growth (IC₅, IC₁₀ and IC₅₀ values). These concentrations and an environmental concentration were then used to expose diatoms under semi-static conditions for 28 days to assess the teratogenic potential of glyphosate and AMPA on diatom morphology. In both experiments, observed deformations were analysed qualitatively (type of deformations) and quantitatively (relative abundance, severity) using morphometric measurements.

2.05.P-We028 Indications of Pollution-Induced Community Tolerance (PICT) of Microphytobenthic Communities in Small Agricultural Streams

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Small streams harbour a variety of communities in agricultural landscapes and are frequently polluted by chemical mixtures from agricultural run-off. The characterisation of these mixtures and their effects on microphytobenthos (MPB) have rarely been studied in agricultural streams. This work suggests a combined approach for analysing chemical mixtures in MPB from field samples and the evaluation of effects on the structural and functional, i.e. photosynthetic performance as well as tolerance patterns of photosynthetic biofilms. 15 streams from agricultural and non-agricultural landscapes were investigated for their chemical and non-chemical stressor profiles (i.e. nutrients, light climate) and MPB was sampled from autochthonous stones as well as from artificial substrates incubated for 6 weeks on site. About 400 chemicals were analysed within the biofilms with a therefore developed chemical extraction protocol using LC-HRMS. The diatom community assembly was assessed by metabarcoding, both from natural substrate as well as from artificial substrate on site. Pollution-induced community tolerance of the biofilms was assessed in a short-term exposure experiment. Our study will present results illustrating biological effects of pollution on photosynthetic biofilms. It could be shown that biofilm communities from 9 out of 10 agricultural sites showed an up to 20 times increased community tolerance. This is one of the effects illustrated in this study deriving from chemical pollution on site. Up to 324 individual chemicals were found in biofilms from agricultural sites, with a significantly higher chemical load in biofilms from agricultural streams than non-agricultural streams. The detailed characterisation of pollution within the biofilms may give further insights into the effect drivers. Our results further open the perspective of biofilms to be used as a cheap and universal tool for effect- as well as for refined exposure assessment.

2.05.P-We029 Clogging LED to Stronger Effects Than Environmental Copper Contamination on Hyporheic Microbial Communities

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The hyporheic zone can be defined as the water saturated sediments below and beside the riverbed. This zone plays a key role in the ecological and biogeochemical river processes such as organic matter decomposition or pollutant degradation. However, anthropogenic activities leading to physical river alterations (e.g. hydroelectric dam) and chemical pollution (e.g. pesticides, heavy metals) can disturb the hyporheic zone functioning. Clogging caused in particular by the erosion of cultivated soils and copper used as a fungicide are generally concomitant in agricultural river catchments. The impacts of these two stressors have been explored independently on microbial communities of the hyporheic zone, but their combined effects are still unexplored. In this study, (1) the effects of clogging on copper distribution in the hyporheic zone and (2) the combined effects of clogging and copper on hyporheic microbial community structure and function were investigated. To this end, we set up a mesocosm experiment using columns filled with sediment and continuously supplied with water during 32 days under controlled conditions to mimic the hyporheic zone and study cause-effect relationships between combined stressors and microbial responses. Four treatments were tested in a full factorial design: (1) control, (2) exposed to copper ([Cu]_{nominal}=191µg.L⁻¹), (3) clogged, (4) clogged and exposed to copper. At the end of the experiment, sediments were collected at four depths of the columns (0-3cm, 4-7cm, 10-13cm, 25-28cm) to determine vertical profiles in copper concentration and microbial structure and functions (e.g. microbial community structure, respiration, denitrification, extracellular enzymatic activities, copper induced tolerance). The results of this experiment showed that copper was filtered mainly in the first few centimeters of the columns, independently of clogging, protecting the microbial communities in deeper layers. In this first centimeters, clogging had greater effects than environmental copper concentration on microbial communities. Whatever the treatment, the interface between surface and hyporheic zone was characterized by a particular microbial community in term of structure, functions and acquisition of tolerance to copper. The information provided by this experiment contribute to a better understanding of the impacts of combined stressors on the hyporheic zone.

2.05.P-We030 Physiological Traits As Biomarker to Assess the Chronic and Acute Exposure of Zn on Mediterranean Barbel

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The responses to the stress caused by anthropogenic alterations and environmental extreme conditions are compensated by the energy trade-offs of an organism to maintain homeostasis. Physiological traits related to metabolism and locomotor behavior are often overlooked to be used as a biomarker of effect in ecotoxicology. In our study we employed metabolic traits (SMR, MMR) and swim capacity (Ucrit) in combination with a battery of antioxidant enzymes (AE). Mediterranean barbel were sampled from Osor river (NE Spain). The concentration of Zn in different reaches of the river ranges from 36 $\mu\text{g L}^{-1}$ to 450 $\mu\text{g L}^{-1}$ implying that its essentiality to act as a micronutrient diminishes. Therefore, chronic test was conducted on wild fish, sampled close to mine effluent (high Zn) and controls were taken from upstream (low Zn). Acute toxicity however was investigated after giving 1 mg L^{-1} of Zn in a controlled environment for 96h. The results of swimming performance showed that chronic exposure did not impact the SMR, but it significantly reduced the MMR. However, Ucrit and AAS were not significantly affected. On the contrary, acute exposure lowered MMR, Ucrit and AAS significantly. The AE response depicted that Zn induced tissue specific and duration dependent AE activities with a higher effect on chronic exposure than the acute. This could be the cumulative effect of Zn in fish tissues followed by oxidative stress due to the long duration, even at lower Zn concentrations. Lipid peroxidation (TBARS) only occurred in the gill and liver in wild fish despite the oxidative stress modulation to avoid it. GSH responses varied with tissue type and exposure duration. It seems that Zn hampered the electron transport chain thus decreased the cellular metabolism and energy acquisition capacity. As a result, the ability of fish to carry out energy intensive activities reduced (low MMR) with a pronounced effect of acute concentration. This in turn gives competitive advantage to the fish from less polluted areas compared to fish from polluted waters, as the studied traits are crucial to determine the energy available to escape predators, capture prey, migrate, select habitat and to reproduce. Our study infers that the use of metabolic traits and locomotor behaviour as a biomarker of ecotoxicity has an ecological value. It is indispensable to understand the role of energy dynamics to understand the repercussion of pollution on the efficiency of functional group to serve the ecosystem.

2.05.P-We031 Laboratory Observations of Oil Induced Sublethal Toxicity Are Reflected in Survival and Reproduction of Tagged Wild Mahi-Mahi (*Coryphaena hippurus*)

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Environmental regulations and impact assessments are based primarily on simplified and controlled laboratory experiments. While such experiments are practical and reproducible, the link between their outcomes and the impacts on wild individuals and populations are rarely verified. To address this shortcoming, we compared laboratory oil-exposures to in-situ observations of wild tagged mahi. Wild mahi were angled from a sport fishing vessel and transferred to a research vessel where oil exposures were performed. In total, 50 wild mahi were tagged with pop-up satellite archival tags (PSATs), with 24 exposed to the water accommodation fraction of Deepwater Horizon source oil and 26 exposed to uncontaminated seawater for a 12-24-hour period prior to release of the tagged fish. Earlier laboratory experiments demonstrated that brief 24-hour exposures in early life stage (ELS) and juvenile mahi led to sublethal impacts on visual acuity, behavioral responses to olfactory cues, and cardiovascular function with consequences for aerobic scope and swimming performance at 1-30 $\mu\text{g?50PAHs/L}$. Notably these effects were predicted from RNAseq experiments on ELS. RNAseq analyses on fin clips obtained from adult tagged mahi prior to release confirmed gene expression changes after exposure to $35.0 \pm 3.4 \mu\text{g?50PAHs/L}$ which were similar to those observed in fish exposed under laboratory conditions, suggesting impairment of cardiovascular function. Wild mahi released after exposure to $35.0 \pm 3.4 \mu\text{g?50PAHs/L}$ for 12-24 hours showed a significantly greater predation mortality during the first 7 days post release. Detection of spawning events from acceleration data documented spawning in control mahi at rates comparable to previous studies. However, over similar periods and locations, wild oil-exposed mahi did not participate in any spawning event. We observed no mortality in tagged mahi during exposure and prior experiments on captive mahi revealed no impact of tagging on spontaneous swimming activity, before, during and after feeding, and showed no reduction in feeding efficiency. The PSAT experiments on wild mahi suggest that sublethal effects observed in laboratory experiments translate to reduced survival and reproduction in wild mahi and further, that even brief low-dose oil exposures of adult fish can cause long term changes that potentially impact population dynamics. This research was made possible by a grant (SA-1520) from The Gulf of Mexico Research Initiative.

2.05.P-We032 Different Biotic and Abiotic Environments Change the Responses of *Lumbriculus variegatus* to Long-Term Exposures to Chemicals

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Environmental risk assessment (ERA) is nowadays based on high acute exposures to a small number of model species in the laboratory using lethal endpoints as observed effects. However, these experiments can hardly simulate the complex conditions of natural environments, where low-level and long-term exposure to chemicals is the day?to?day life. These exposures cause several subcellular changes within the organisms, leading to adverse effects in the long run, which short-term experiments can not detect. For the implementation of chronic exposures in the ERA, new non-standard endpoints need to be investigated. This is the aim of the Marie Curie International Training Network CHRONIC. As part of the CHRONIC network, the present work aims to evaluate

the consistency of chemically induced initiating events (lower level of biological organisation) and individual-level responses (physiology and behaviour) in *Lumbriculus variegatus* under long-term, low-level exposures, along with combined stressors' exposures (chemical, abiotic and biotic stressors). For this purpose, a chemical model will be chosen based on a first screening approach using standardised short term technical guidelines. Physiological markers represent a useful tool in ERA, as they represent initiating key effects measured at the subcellular level to sublethal exposure. Biomarkers can show effects before irreversible changes occur within the organisms and are considered fast and sensitive responses. The chosen biomarkers for the present study represent the xenobiotic metabolism (e. g. glutathione-S-transferases, sulfotransferase) and the defence response (e. g. catalase, lipid peroxidation rate). Additionally, biomarkers representing the general condition of the organisms, including the energy budget (e. g. glycogen, carbohydrate, lipid contents), will be used for the approach to link subcellular effects to higher biological levels. All biomarkers will be combined in an Integrated biomarker response (IBR). The behavioural response of the organisms and genotoxicity, as well as the dynamic energy budget model and adverse outcome pathways, will be used to link the subcellular response to higher biological levels.

2.05.P-We033 Comparative Study of the Ecotoxicity in Water and Soils of 4 Hydrolates and 2 Natural Products of Plant Origin

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Plant-based products are frequently considered as efficient and more environmentally friendly alternative against synthetic products for many applications such as biopesticides or antimicrobials. In fact, many of them are already commercialized under “green” labels. Nevertheless, few research have analysed their ecotoxicity when they are released and accumulate in the environment. We conducted a comparative study of the ecotoxicity in water and soil for four hydrolates obtained by semi-industrial vapor-pressure extraction from three selected aromatic plant species: (domesticated *Artemisia absinthium* (Teruel, Spain), *Dittrichia graveolens* (Ciudad Real, Spain), and experimentally pre-domesticated *Lavandula luisieri* (Toledo, Spain)); two plant-based products (citronellol and tannic acid) and a commercial synthetic pesticide (fipronil). In order to determine ecotoxicity in the environment, four indicator organisms were selected: the invertebrate *Daphnia magna* and the bacterium *Vibrio fischeri* as aquatic organisms and the earthworm *Eisenia fetida* and the plant *Allium cepa* as soil organisms. In addition, the study of mesocosms samples exposed to the tested products allowed to evaluate the global impact in fluvial periphyton and microbial communities of both aquatic and soil ecosystems. Our results concluded that the *S. montana* hydrolate is the most toxic for *V. fischeri* and *A. cepa*, being also the one that most affects to the fluvial periphyton and the mesocosm of water and soil microorganisms (on which pesticide fipronil has no effect). *A. absinthium* hydrolate is was especially harmful to *D. magna* and *E. fetida*; while *D. graveolens* hydrolate mainly affects *E. fetida* and *A. cepa*. Finally, citronellol and tannic acid showed higher LC₅₀ values for *D. magna* and fluvial periphyton than fipronil, although they have had higher antimicrobial activity against microbial communities in rivers and soil than the synthetic pesticide. Without denying the reasonable possibility that plant-based products biodegrade more easily than synthetic pesticides, and they might generate less bacterial resistance, systematic monitoring of their ecotoxicity should be required before they are proposed as viable alternatives to synthetic products since it has been shown that their natural origin does not imply harmlessness to the environment. *The authors thank the financial support of Gobierno de Aragón: Departamento de Ciencia, Universidad y Sociedad del Conocimiento (Group E39_20R).*

2.05.P-We034 First Look Into the Use of Fish Scales As a Medium for Multi-Hormone Stress Analysis

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Cortisol (F) is the main glucocorticoid and a primary mediator of the physiological stress response in fishes. While the quantitation of secreted cortisol provides some indication of the state of stress within an organism, the use of multi-hormone analyses may be capable of uncovering further information. Dehydroepiandrosterone (DHEA) is an androgen and precursor steroid with actions that oppose those of cortisol in mammals. Currently, the potential role of DHEA in the stress response in fish is unclear; however, in humans and other vertebrates high ratios of cortisol to DHEA are considered indicative of chronic stress and an increased allostatic load. The means by which DHEA negates the effects of cortisol in mammals is thought to occur in part via changes in the metabolism of cortisol to its inactive metabolite cortisone (E). The quantitation of F as well as DHEA and E could therefore provide a more complete picture of the overall state of stress in fishes. While the quantitation of glucocorticoids to assess stress in fishes is often accomplished using plasma or serum, the use of fish scale hormone concentrations in the evaluation of long-term stress presents some unique and useful features. Scales incorporate steroid hormones as they grow and thus present an integration of hormones secreted over lengthy periods of time rather than a single time point as is characteristic of other media. Additionally, scale hormone concentrations are most likely unaffected by brief increases in stress incurred upon capture, a problem often encountered when sampling blood. As DHEA and E have yet to be quantified within the fish scale the first objective of this study was to develop a sample processing protocol for the extraction and quantitation of F, E and DHEA. Following this, we induced a state of long-term stress in goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*) to determine changes in scale and circulating F, E and DHEA concentrations. After 14 days of randomized daily physical stressors, scale concentrations of F, E and DHEA were elevated compared to unstressed controls in both fish species, however these elevations were not reflected in all serum samples. This study thus supports the use of fish scales in place of other media when assessing long-term stress in teleost fish.

2.05.P-We035 Potential Impacts of Sacrificial Anodes on the Health Status of the Black Scallop *Mimachlamys Varia*: Laboratory and Field Study

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Coastal areas are important interface environments between marine and terrestrial ecosystems and are populated by abundant living communities. They are generally areas with high levels of economic activity, particularly linked to the presence of ports areas (seaport, fishing and marina). These port areas have developed marine ecosystems in their basins, which may be subject to various forms of contamination resulting from port activities. Heavy metals in particular may be at issue. Indeed, they may come from roadways, river discharges, careening stations, but also from cathodic protection equipment. To date, few biomonitoring studies have examined the potential impact of cathodic protection on biodiversity in port areas. This phenomenon slows down the corrosion of steel structures caused by sea water thanks to sacrificial anodes. In fact, these anodes will corrode in place of the structure they protect and thus release the metals they contain (aluminum, zinc and indium-based alloys) into the water. Large quantities of anodes are therefore necessary to protect submerged infrastructures, and consequently the quantity of corrosion products resulting from the degradation of anodes seems considerable. Studies were therefore carried out in 2021, both in port areas and in the laboratory, in order to observe whether sacrificial anodes have an impact on the health status of black scallops through several biomarker responses. Described in the literature as a sentinel species in the marine environment, the black scallop is a filter feeder with a sedentary lifestyle, allowing it to bioaccumulate and tolerate large quantities of contaminants in its body. It thus seemed the most suitable species for this study. A first study was set up in the seaport and the marina of La Rochelle, on sites with old anodes and sites with new anodes, accompanied by a reference site without anodes and without influence from port activities. A second study, carried out in the laboratory, aimed to reproduce the same conditions as the first study with regard to anodes, but without the influence of port activities. The first results showed that in a port areas the different activities (trade, careening) and the meteorological conditions greatly influence the data and that the potential effect of sacrificial anodes is not visible. The study in a controlled environment, the results of which are still being analyzed, should make it possible to overcome these constraints and provide new answers.

2.05.P-We036 Methodological Adaptation of Mesocosms for Complex Substances Like Paraffin Oils : Results of Environmental Impact Assessment for Insecticide and Acaricide Uses in Crop Protection

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In order to assess the environmental impact on aquatic ecosystems of paraffin oils (used as insecticide and acaricide for crop protection), specific experimental facility and equipment must be used to overcome the challenge of the hydrophobicity/low solubility of that kind of substances. A study was conducted between July and December 2020 at the Pilot Rivers platform at Lacq (France) in outdoor freshwater open-stream mesocosms, fed with 200 m³/h by Gave de Pau, which are a very ecologically relevant system. The principle of this experimentation was to expose colonized ecosystems from artificial streams during 28 days (followed by 28 more days of recovery) to defined concentrations of formulated paraffin oil, in order to observe and measure the biological responses in time of different organisms and communities of organisms (chlorophyll-a, milfoil growth, diatoms, litter breakdown, benthic macro invertebrates, Gammarus), and to determine the magnitude and significance of the effects in comparison to control ecosystems. The continuous treatment was applied at higher concentrations than recommended for paraffin oil applications at full scale for plant protection, so that the results of the study can be considered as overconservative. This study was not designed to build a full dose-response relationship, but to assess 2 concentrations of the paraffin oil and determine the highest tested concentration with No Effect. From the results regarding the experimental design, it is concluded that there is no risk for aquatic environment at the tested concentration of 5 mg/l for the formulated product (EC, 97% paraffin oil). The evaluation of the data was conducted in agreement with the EFSA guidance including Minimum Detectable Differences calculation (Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters EFSA Journal 2013;11(7):3290).

2.05.P-We037 Interactive Effects Between Light and Temperature During Glyphosate Degradation in River Biofilms

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Global change is expected to alter environmental conditions driving the structure, diversity and functions of biological communities in aquatic ecosystems. Among ecological functions, we investigated how temperature and light variations affected biofilm's capacity to attenuate the herbicide glyphosate from running waters. With this aim, natural biofilm communities were acclimated to two water temperature regimes (Ambient = 19-22 °C, Warming = 21-24 °C) and three light conditions (Dark = 0 Lux, Intermediate = 600 Lux, High = 1600 Lux) in a factorial design experiment in the laboratory. After four-weeks acclimation, all biofilms were contaminated with glyphosate (50 µg/L final concentration) and their capacity for glyphosate dissipation was monitored during 21 days. The transformation of glyphosate in the warming condition was faster at high light ($DT_{50} = 6.36$ days) comparing to intermediate light and dark conditions (8.53 and 9.49 days, respectively), though differences in light effects were not observed at the ambient temperature condition (11.35, 10.13 and 8.41 days, respectively). Biofilms transformed glyphosate into aminomethyl phosphonic acid (AMPA), but were not capable to completely mineralize the molecule suggesting that the latter will tend to accumulate in the environment. Light was a structuring factor of bacterial communities diversity subjected to warm temperatures, *Burkholderiaceae* taxa were characteristic of this condition (IndVal = 0.98, $P < 0.005$). Light also influenced the structure of eukaryotic communities subjected to both ambient and warm conditions. Protozoan (*Flamella* sp.) and diatoms

(*Fistulifera sp.*) were indicator taxa from high light conditions in eukaryotic communities. Light and temperature variations are important factors regulating microbial communities capacity for glyphosate removal in river ecosystems.

2.06 Multi-stressor ecotoxicology in aquatic ecosystems in a rapidly changing climate (Part I)

2.06.T-01 The Impact of Consecutive Heat Wave and Pesticide Exposure on Two Congeneric Damselfly Species and Their Gut Microbiome

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Freshwater organisms are often exposed to both toxicants and natural stressors, whereby it is key to assess the combined impact of toxicant exposure under warming. Both heat waves and pesticides are frequently occurring stressors in aquatic ecosystems and are expected to increase under global warming. Since their transient nature, and because organisms are often sequentially exposed to these stressors, it might be expected that negative effects of a pesticide could be increased due to previous exposure to a heat wave, if the heat wave reduces the energy budget to deal with the pesticide. Therefore, we examined the effect of consecutive exposure to a heat wave (absent and present) and a pesticide (solvent control and 2 µg/L chlorpyrifos) on two life history traits (mortality and growth rate) and three physiological variables (acetylcholinesterase activity, malondialdehyde and cellular energy allocation) in two congeneric *Ischnura* damselfly species (*I. elegans* and *I. pumilio*). Of a subset of larvae, the gut microbiome was dissected to analyze the effects of the stressors on the microbiome community composition and whether these differed between species, and covary with the response patterns observed for the life history and physiological variables (analyses in progress). Larval life history was only negatively impacted by exposure to the heat wave, whereas the physiological variables were altered by both stressors. Moreover, we found that *I. pumilio* had a higher energy budget than *I. elegans*, which allowed them to be better able to cope with the increased energetic demands under stressor exposure. We did not find any evidence of higher negative effects of the pesticide after exposure to a heat wave. Potentially, cross-tolerance occurred: by activation of the defense system under heat wave exposure, larvae might have been able to better withstand subsequent pesticide exposure, and thus do not show an increased negative response to the second stressor. Our results highlight that life history traits may not always show the combined impact of stressors on organisms, and that physiological variables may be more sensitive biomarkers.

2.06.T-02 Ecotoxicological and Biochemical Effects of Two Pesticides, Single and Combined, on the Marine Diatom *Thalassiosira weissflogii* - the Impacts of Climatic Changes

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In the last decades, climatic changes have been a great concern among the scientific and political communities. Nowadays, climatic changes effects have been reported associated to extreme climatic events like the raise of temperature, floods and droughts with impacts in several activities and in the ecosystems. The exponential increase in world population and growth in food consumption are placing unprecedented demands on agriculture and natural resources, what enforces to an intensive use of fertilizers and pesticides to suppress food production requirements. So, it is crucial to understand the harmful effects of pesticides in aquatic systems, in a context of climatic changes. In this work was considered the effects of the pesticides copper sulphate and oxyfluorfen on the marine diatom *Thalassiosira weissflogii*, at three temperature scenarios (15°C, 20°C and 25°C). This work intends to: a) evaluate the growth rate of *T. weissflogii* exposed to both pesticides, single and combined, at 15°C, 20°C and 25°C; and b) determine the biochemical impacts, namely the fatty acids (FA) profiles, after the exposure to both pesticides at 15°C, 20°C and 25°C. Results showed that this species is highly sensitive to oxyfluorfen, with EC50 values in the µg/L range. A greater growth rate of *T. weissflogii* was found at higher temperatures (20°C and 25°C) than at lower temperatures (15°C). However, increased temperature leads to increased sensitivity of the species to both compounds (at 25°C, EC50_{Copper sulfate}=98µg/L; EC50_{oxyfluorfen}=2.46 µg/L). Interactions between compounds showed a marked antagonism at 20°C and 25°C, but at 15°C a synergistic effect was observed at low concentrations of oxyfluorfen and at high concentrations of copper sulphate. Regarding on the effects on FA profiles, the organisms exposed to highest temperatures (20°C and 25°C), showed to be more affected by the pesticides (dissimilarity between control and combined pesticides at 20°C=92 % and at 25°C=79%), than the ones exposed to the lowest temperature (15°C) (dissimilarity between control and combined pesticides=14.98 %). These results raise concern over the potential effects of climate change, considering the predicted increase in temperature and the presence of mixtures of these pesticides in the environment. Under these conditions, this species will face decreased growth rate and impaired nutritional value, which will likely impact the trophic food webs and, thus, the structure of marine communities.

2.06.T-03 Long-Term Effects of Warming and Pyrene on *Calanus hyperboreus*

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The copepod *Calanus hyperboreus* is a key species in the Arctic marine ecosystems being vital lipid-rich food for Arctic fish, birds, and mammals. However, cumulative long-term effects of warming and pollutants on *C. hyperboreus* population success are of concern. To address this issue, we conducted a one-year experiment using *C. hyperboreus* collected in March 2021 from the upper 400 m in the deep Arctic Ocean northeast of Svalbard. *C. hyperboreus* females were assigned individually in 250-ml glass bottles filled with 200 ml of the filtered seawater at 1 or 5°C (n = 40 for each temperature). After two weeks, 20 females of each temperature treatment were exposed to one pulse of pyrene (200 nM). In May, which was the algal bloom period, females were fed six times per week (Mon-Fri) with *Rhodomonas salina* (~500 µg C L⁻¹). The survival, egg production, hatching success, and

grazing rate (faecal pellet production) were quantified through the productive season from 31 March to 30 September 2021. The lipid content was measured in May, July, August, and September to assess how females recovered from reproduction. No short term treatment effects were detected on survival or egg production after just 14 days, but over the longer course of the year, the long-term experiment females exposed to pyrene had both lower survival and lower faecal pellet production than controls, particularly at 1°C. Survival and grazing rate was generally higher at 5°C than at 1°C, suggesting that a +4°C warming is not critical for *C. hyperboreus*. Surviving females were kept and are currently overwintering at 1°C in filtered seawater, starting from November 2021 until February 2022. Survival and gonad development of females is checked weekly. Final egg production and potential egg hatching success will be quantified in January-March 2022. This study will provide unique information on long-term oil pollution and warming effects on *C. hyperboreus*, a key species in high-Arctic marine food webs.

2.06.T-04 Influence of Salinity on Pyrethroid Toxicity: An Analysis of Neurotoxicity and Osmoregulation in a Model Estuarine Fish (*Menidia beryllina*)

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Climate change is causing changes in precipitation patterns and as well as increased sea levels. These alterations are linked with increased salinity in estuaries, making the potential differences in toxicity across a salinity gradient a topic of increasing interest in assessing risk to estuarine species. As ionic concentration increases the log K_{ow} and water solubility of a compound has been shown to increase and decrease respectively. Pyrethroid insecticides are commonly used in agricultural, industrial, and household settings and have been detected in watersheds globally. Several recent studies have shown that pyrethroid toxicity can change across a salinity gradient. Early life exposures in fish to pyrethroids has been found to cause toxicity at environmentally relevant concentrations and alter behavior, reproduction, and gene expression. Therefore, Inland Silversides (*Menidia beryllina*), a commonly used euryhaline, model fish species, were exposed from 5 days post fertilization (approximately 1-day pre-hatch) for 96 hours to six pyrethroids: bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, esfenvalerate, and permethrin. Exposures were conducted at three salinities relevant to brackish, estuarine habitat (0.5, 2, and 6 PSU) and 3 concentrations, either 0.1, 1, 10, and/or 100 ng/L, determined from previous experiments to be sublethal and environmentally relevant. After exposure, Inland Silversides underwent behavioral assays and were subjected to a dark and light cycle to determine behavioral toxicity.

Additionally, Inland Silversides exposed to bifenthrin, cyfluthrin, and cyhalothrin at 6 and 10 psu and 1 ng/L, were placed in clean water and are being reared until reproductive age. F0 larvae, F0 adults, and F1 larvae will undergo behavioral analysis and gene expression analysis to inform on the potential multigenerational effect of pyrethroids. These results indicate that there may be different behavioral responses to pyrethroids depending on exposure salinity and concentration. There was developmental toxicity observed from cypermethrin exposure at 0.5 PSU, but not from other compounds. This suggests behavioral differences are related to neurological effects rather than developmental impacts. Results show different behavioral responses in each pyrethroid at different salinities. These data will provide knowledge to managers and environmental planners to help further protect threatened and endangered fishes in estuarine and bay regions.

2.06.T-05 Do Short-Chain Chlorinated Paraffins Have Endocrine Disrupting Potential? Molecular and Population-Based Investigation in the Rotifer *Brachionus calyciflorus*

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Rotifers, one of the most important zooplankton groups, play a vital role in nutrient cycles by feeding the upper levels of aquatic food chains. Their complex life cycle, alternating between periods of parthenogenetic and sexual reproduction which produces diapausing cysts, offers multiple endpoints with varying sensitivities to contaminants. This makes them attractive models for ecotoxicology, which is why the freshwater species *Brachionus calyciflorus* was chosen to study the impact of short-chain chlorinated paraffins (SCCP), persistent organic pollutants with known endocrine disrupting effects, on zooplankton. To this end, their demographic parameters and the mRNA levels of three endocrine receptors were measured after exposure to environmentally realistic SCCP concentrations (10-100-1000ng/L). A 4-day reproductive test on *B. calyciflorus* evaluated SCCP impact on the intrinsic population growth rate (r), mixis (M), fertilization (F), cyst production, and hatching rate. The mRNA levels of the endocrine genes retinoid X receptor (RXR), membrane-associated progesterone receptor (MAPR), and retinoid A receptor (RAR) were measured after 6-24-48-72-96 hours of exposure. Furthermore, both assays were carried out at two temperatures, 20°C and 25°C, to determine if the known effect of temperature on *B. calyciflorus* reproduction would influence SCCP effects. Results showed that multiple demographic values of *B. calyciflorus* are significantly impacted by SCCP exposure. Indeed, at both temperatures, M was significantly higher than control, while F was lower, indicating possible issues with sexual reproduction. However this did not affect cyst production and hatching rate. The only factor that differed between temperature treatments was r, which was significantly lower than controls at 100ng/L of SCCP only at 20°C. The three studied endocrine genes were all affected by SCCP exposure with significant increases in mRNA levels compared to controls taking place after 72h, which is the point where sexual reproduction happens in the reproduction assay. In conclusion, environmentally relevant SCCP exposure affects numerous reproductive factors in *B. calyciflorus*, as well as transcription levels of endocrine genes which are believed to influence reproduction. These effects did not affect cyst production and hatching. A 5°C difference between treatments seemed to mainly influence SCCP effects on two elements: parthenogenetic reproduction and the RXR gene.

2.06 Multi-stressor ecotoxicology in aquatic ecosystems in a rapidly changing climate (Part II)

2.06.T-06 Adaptation to Pesticides and Associated Fitness Costs Under Single and Multi-Stress Conditions

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Under global change scenarios, multi-stress conditions may occur regularly and requires adaptation. However, the adaptation to one stressor may reduce performance due to fitness costs and long-term effects of adaptation. Here we investigated the ecological consequences of adaptation under multiple stress and long-term consequences of pesticide exposure to ultra-low concentrations in the widespread crustacean *Gammarus pulex*. Under optimum temperature, *G. pulex* from agricultural streams were considerably more tolerant to pesticides as compared to the reference populations. Here we assume that the increased tolerance in agricultural populations is the combination of acclimation, epigenetic effect and genetic evolution. After experimental pre-exposure to very low concentration (LC₅₀/1000), reference populations showed increased pesticide tolerance. In contrast, pre-exposure did not further increase the tolerance of agricultural populations. Moreover, these populations were more sensitive to elevated temperature alone due to the hypothesized fitness cost of genetic adaptation to pesticides. However, both reference and agricultural populations showed a similar tolerance to the combined stress of pesticides and warming due to stronger synergistic effects in adapted populations. As a result pesticide adaptation loses its advantage. The combined effect was predicted well by the Stress Addition Model (SAM), developed for predicting the synergistic interaction of independent stressors. Long-term consequences of increased insecticide tolerance were characterized by significantly reduced survival, per capita growth and mating when organisms were cultured under pesticide-free conditions in the laboratory for three months. We conclude that under multi-stress conditions, adaptation to pesticides reduces the general stress capacity of individuals and trade-off processes increase the sensitivity to additional stressors. This causes strong synergistic effects of additional stressors on pesticide adapted individuals. The exposure and adaptation are also associated with impaired performance which potentially affects ecosystem functions such as leaf litter degradation. These long-term impairments need to be considered in deriving safe concentrations.

2.06.T-07 A Heat Spike Overrides Pesticide Tolerance: A CASE Study in *Daphnia magna*

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Two important anthropogenic stressors that cause biodiversity loss in aquatic ecosystems are global warming and agricultural insecticides. Not only the mean temperature, but also temperature extremes are predicted to increase under global warming. Pesticide exposure can cause severe sublethal and lethal effects and therefore poses a strong selection pressure, leading to the evolution of pesticide tolerance. Despite making it possible for organisms to survive in a polluted environment, tolerance also comes with costs, most often due to energetic constraints. One such highly relevant, but rarely studied cost of tolerance is an increased sensitivity to heat. Building on an experimental evolution trial during which tolerance towards the pesticide chlorpyrifos evolved in *Daphnia magna*, we compared the heat sensitivity of chlorpyrifos-tolerant and non-tolerant clones, whereby both lethal and sublethal traits (reproduction) were investigated. All clones were exposed to one of the six combinations of four days exposure to chlorpyrifos (solvent control, 0.5 µg/L or 0.65 µg/L) followed by four days exposure to a heat treatment (20 °C vs 30 °C). Survival was defined as the percentage surviving individuals at maturity. To get an estimate of the reproductive outcome, both time to the release of first and second broods and the brood sizes were determined. We observed a strong cost of chlorpyrifos tolerance in terms of a lower heat tolerance, both for survival and traits related to reproduction. Most importantly, these heat-induced costs paid by the chlorpyrifos-tolerant clones did not disappear in the presence of the pesticide. Intriguingly, the opposite was true: when combined with exposure to chlorpyrifos, the heat spike caused the highest mortality (at the highest pesticide concentration), delayed reproduction and reduced the number of offspring the most. This suggests that heat spikes might offset pesticide tolerance. In the long term, this might mean that in a warming world where heat spikes will become more frequent, pesticide-tolerant clones might suffer more, possibly leading to shifts in the population composition and the population tolerance level.

2.06.T-08 Single and Combined Effects of the Herbicide Terbutylazine and Increased Temperature on Synthetic Periphyton Structure, Function and Microbial Composition

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Phototrophic biofilms, also known as periphyton, are microbial freshwater communities that drive crucial ecological processes in streams. Periphyton is influenced by a multitude of biotic and abiotic factors, which may alter its composition and the ecological functions it provides. Due to the complexity of natural periphyton, understanding species dynamics is challenging, yet fundamental to gain mechanistic insights into the impacts of multiple stressors such as chemical pollution under the conditions of climate change. To address this challenge, we used a fully defined but diverse, and highly reproducible synthetic periphyton, consisting of an assemblage of at least 22 phototrophic species (13 diatoms, at least 5 green algae, 4 cyanobacteria). We examined the single and combined effects of increased temperature (17°C vs 20°C) and four levels of the herbicide terbutylazine (0, 1, 10 and 100 µM) on the 3D-structure, function and microbial composition of the synthetic periphyton. Our results show that both temperature and 100 nM terbutylazine strongly impacted community composition and microbial succession. On the other hand, functional response of the entire community remained intact upon exposure to stressors, although the relative abundances of multiple species were strongly impacted. Importantly, we were able to examine precisely how each species within the community responded over time to the tested stressors, singly and in combination. Species-level resolution within the community provided an accurate understanding of how species interactions affect the community properties at the functional and structural levels when multiple stressors occur. Overall, our synthetic periphyton is a powerful tool to perform mechanistic studies on periphyton

structural and functional responses, as well as on species propagation, potentially to any biotic and abiotic stressor and their combinations. Indeed, our results provide strong evidence that such a synthetic periphyton, grown under controlled conditions, enables to overcome the complexity of its natural counterpart and to test for various mechanistic hypotheses via targeted manipulation.

2.06.T-09 Future Climate Change Effects of Reoccurring Heatwaves and Elevated Temperature Fluctuations Towards Chemically Stressed Freshwater Ecosystems

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Temperature alterations in consequence of future global climate change (GCC) will affect aquatic ecosystems, such as lentic freshwaters. Latest climate assessments predict higher mean temperatures and an increased severity and frequency of extreme weather events, such as heatwaves or warm spells in the future. The impacts of these natural pressures are, yet, unknown due to complex interactions with other anthropogenic stressors (i.e. chemicals), causing potential alterations in the environmental fate of these ubiquitous compounds. At present, the combined effects of nonchemical GCC-related stressors and chemicals on freshwater ecosystems continue to be understudied. While the majority of multiple stressor experiments observed responses of single species, only few experiments have been conducted at the more complex levels of population or community considering intra- and interspecific interactions at the same or adjoining trophic level. Besides that, studies which include daily temperature fluctuations and thus natural variability are very scarce. However, more severe effects on species under chemical stress were observed under changing thermal conditions when compared to constant, elevated temperature regimes. Therefore, we concluded that ecosystem complexity and the variability in temperature are crucial aspects when studying chemical effects under environmentally relevant climate change scenarios. Our goal was to study single and combined effects of GCC scenarios on zooplankton-dominated freshwater ecosystems under chemical stress. Temperature variability and extreme events were operated by the *Transportable temperature and heatwave control device (TENTACLE)*. All temperature scenarios were replicated five times in the presence or absence of the fungicide carbendazim in indoor freshwater microcosms. We studied ten endpoints over 48 days to comprehend changes in species dynamics, community structure, and ecosystem functioning. Results revealed a significant decline in the abundance of macro- and micro-zooplankton species after reoccurring heatwaves, elevated temperatures, and chemical stress ($F=0.6$, $p=0.002$). Zooplankton community analyses presented rotifers having highest tolerance compared to copepods, while cladocerans showed lowest tolerance towards combined stress. Additionally observed multiple stressor effects were bidirectional, depending on the trophic level and endpoint under investigation and thus highlighting the need for further research.

2.06.T-10 Too Cold and Too Hot Temperatures Increase Pesticide Toxicity

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The majority of studies on the combined impact of pesticides and temperature only considered a limited number of temperatures, typically including an intermediate 'room' temperature and a high temperature. This only captures some of the thermal range animals experience in natural populations. Although exposure to both colder and extreme high temperatures, and to daily temperature fluctuations (DTF), can be perceived as stressful, these are less considered in ecotoxicology. One powerful and conceptual tool to predict organismal responses and the fate of populations to climate change is the thermal performance curve (TPC). Despite their potential to also improve our insights in the fate of populations to warming in polluted world, studies that explicitly considered how TPCs are affected by pesticides are rare. We tested the effects of pesticide exposure and DTF on the latitude-specific thermal performance curves of the damselfly *Ischnura elegans*. While chlorpyrifos did not affect the life history traits at the intermediate temperatures (20, 24 °C), chlorpyrifos became toxic (caused lower survival and growth) at the warmer temperatures (? 28 °C), despite the higher chlorpyrifos degradation rates at these temperatures. At the extreme warm temperatures, AChE activity showed the strongest chlorpyrifos-induced inhibition, which contributed to the observed toxicity patterns at these temperatures. At the highest temperature the pesticide-induced toxicity was further magnified by DTF. Remarkably, chlorpyrifos also became toxic (reduced growth) at the colder temperatures (12, 16 °C). Overall, for all response variables, pesticide exposure resulted in more concave-shaped TPCs compared to the solvent control. By taking a thermal performance curve perspective, our study could identify different toxicity patterns at low, intermediate and high-to-extreme temperatures. This is important for pesticide risk assessment as in natural populations organisms may be exposed to pesticides not just at the advocated constant ecotox test temperature, but may regularly encounter a wide range of temperatures. Our results indicate that making predictions on the fate of populations under future warming based on TPCs, may be biased when ignoring pesticide exposure. This highlights the importance of studying pesticide toxicity across a range of temperatures to make more realistic predictions both about the impact of (i) pesticides in a warming world and (ii) warming in a polluted world.

2.06 Multi-stressor ecotoxicology in aquatic ecosystems in a rapidly changing climate (Virtual Only)

2.06.V-01 Application of Effect Based Methods in the Health Impact Assessment in Italy

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The Health Impact Assessment (HIA) is a procedure aimed at protecting the health of the populations exposed to the impacts caused by large industrial enterprises. These enterprises (e.g. steel plants, petrochemical refineries) can potentially release and discharge several contaminants included in the industrial cycle. The legislations for the protection of air, soil and water quality

contain only short lists of chemicals and the potential effects of other contaminants and the mixtures are not taken into account. For this reason in Italy it has been decided to recommend to the enterprises to apply also effect based methods in the scoping (*ante operam*) and in the monitoring phase of an HIA. Effect Based Methods have been applied in different multistressors scenario (included climate changes) and can represent a valid support to the chemical analysis because they can give informations on the potential effects (e.g. mutagenicity, neurotoxicity, cardiotoxicity, embryotoxicity) caused by the chemical pollution included mixtures in the aquatic and terrestrial ecosystems. The effect based methods in the context of an HIA have been applied and proposed in different areas in Italy where the new building or an upgrading of industrial plants is foreseen. Ten industries have already applied the ecotoxicological assessment in the ante-operam phase submitting a complete monitoring plan. The EBM applied are mainly acute and chronic aquatic and terrestrial bioassays in vivo (e.g. test with earthworms, algae, crustaceans, fish embryos) and ecogenotoxicological assays such as the Ames test and the micronucleus tests. The ecotoxicological assessment may differ on the basis to specific local situations and on additional information acquired on the site and testing should be conducted according to the main national, international guidelines or validated protocols. We think that the application of EBM in an HIA with the role of early warning system and screening can guarantee a better level of protection for human health and ecosystems and at same time can address the industries to adopt the best available technologies to reduce or eliminate chemical emissions and releases.

2.06.V-02 Evaluation of the Phototoxic Effect of Microplastics and Avobenzone Using *Daphnia magna*

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Plastics are used in various fields and their production and consumption are steadily increasing. In disposal process, plastics discharge microplastics of various shapes and sizes. Microplastics can enter the aquatic environment by diverse pathway, and they can adsorb many pollutants. One of a pollutant in aquatic environment is UV filter. UV filters are contained in sunscreen and personal care products to prevent negative effects from the ultraviolet (UV). Particularly in summertime, UV filters can be emitted directly by increasing of water activities. Avobenzone is a commonly used UV filter, it can absorb UVA (320–400 nm) wavelength. When microplastics and avobenzone combined, it can be more toxic effects on aquatic organisms. So, this study aimed to observe combined and phototoxic effects of microplastics and avobenzone on aquatic organism *Daphnia magna*. Acute toxicity test was performed for 48 hours. After 48 hours, significant immobilization rate was observed both non-UV and UV irradiated exposure groups. And UV irradiated groups were not growth well. Combined and phototoxic seems induce more toxic effects on *D. magna*. This study is the first case to report the effect of microplastics and avobenzone on the aquatic organism *D. magna*. These results could be used to understand the effects of UV filter and microplastics in aquatic environment, and stricter regulations are needed. *Acknowledgement- This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2020R1A2B5B02001734)*

2.06.V-03 Legacy Contaminant in a Future Ocean: Effect of Mercury on Cuttlefish Behaviour Under Elevated pCO₂

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Among trace elements, mercury (Hg) is found in the marine environment under both inorganic and organic forms, mainly methylmercury (MeHg). This latter is well known for its efficient bioaccumulation in biota, biomagnification along food webs, and high neurotoxicity. Despite their central place in the trophic chain and their short life span, cephalopods can display Hg concentrations up to 1 µg.g⁻¹ dry weight in their tissue. Experimental evidence demonstrated that assimilated MeHg by the trophic route is the predominant pathway of total Hg bioaccumulation in the common cuttlefish *Sepia officinalis*. In addition, the cephalopod brains are target organs of Hg accumulation raising the question of its toxic effect on nervous system. Moreover, atmospheric emissions of carbon dioxide (CO₂) increases induce ocean acidification (OA) which affects the physiology and altered neurosensory capacities of marine organisms. However, information on Hg and OA toxicity in cephalopod are still scarce, even though these molluscs have a developed central nervous system, which could be affected by both of these stressors. In this context the main objective of this work is to assess the impact of these two neurotoxic (Hg and pCO₂) on behavioural and cognitive performance of cuttlefish. Juvenile were raised in control condition, high pCO₂ and/or dietary MeHg exposure and passed several behaviour assays (predation/anti-predation abilities and lateralisation acquisition) during the first month of life. Predatory activities were not impacted by Hg and/or pCO₂. However, the acquisition of visual lateralisation in juvenile exposed to high pCO₂, Hg or both combined was disturbed. While 60% vs. 10% of control cuttlefish use their left vs. right eyes, respectively, in a vigilant situation, this proportion changed to 35% vs. 35%, respectively, for cuttlefish exposed to stressors and thus demonstrated a reverse lateralisation. In addition, it was also observed that the cuttlefish exposed to single or combined stressors progressively lost their camouflage capacities during the 1st-month period. Scarce information on fish and mammals highlights Hg disruptions in cellular processes including synaptic function, excitability, ion regulation, and protein synthesis. Moreover, OA is known to altered GABA_A receptor function. Here, the GAD gene expression was quantified by *in situ* hybridization in brain of tested individuals and interpreted with respect to behavioural and cognitive performances.

2.06.V-04 Multi-Metallic Stress and Global Change at Contrasted Reproduction Cycle Stages: Combined Effects in a Sentinel Freshwater Mussel, *Dreissena polymorpha*

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Global change issues constitute new challenges in ecotoxicology. Although no consensus was established between the occurrence of extreme events and global changes, the frequency and the magnitude of heat waves is still increasing. The basal metabolic activity of ectotherm organisms could be strongly affected by quick temperature variations, limiting thus their defence capacity facing to additional stresses. This study aimed to assess the combined effects of a multi-metallic stress and recurrent heat waves in a freshwater bivalve, *Dreissena polymorpha*, during contrasted reproduction cycle steps. Mussels were exposed to an environmental concentration of a nickel-chromium mix solution ($20 \mu\text{g.L}^{-1}$ and $5 \mu\text{g.L}^{-1}$, respectively) for 40 days. A gradual increase of the temperature ($+15^\circ\text{C}$) was weekly performed in exposed media for 24 hours. The same experimental design has been set up during the reproduction (May-June) and the rest (November-December) periods. Key metabolism functions were targeted along the biological organisation levels through a panel of markers involved in the energy metabolism, defence mechanisms and cellular damage. Different patterns of physiological responses were highlighted between both sexes and the two reproduction cycle stages. An effect of recurrent heat waves was observed in females' osmolality while males' one was only impacted by the multi-metallic stress. Mussels exposed to the combined stress showed a decrease of their global metabolic activity associated with an increase of defence mechanisms. This study pointed out the need to include global change issues and temporal variations for further ecotoxicological investigations.

2.06.V-05 The Use of Fatty Acids to Assess Nutritional Quality of Natural and Urban Gulls' Diets Relating to the Ingestion of Debris Materials

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Urban habitats offer predictable anthropogenic food sources which attract opportunistic and generalist animals, such as gulls. These species are known to exploit urban areas and landfills taking advantage of accessible, abundant, and diverse food sources, allowing them to reduce foraging time and energy expenditure. Human-derived food, however, is characterized by a poorer nutritional quality when compared to the typical natural food resources; additionally, foraging in urban areas may increase birds' susceptibility of ingesting anthropogenic debris materials, both with unknown physiological consequences for urban and landfill dwellers. In this study, we compared fatty acids (FA) composition of two opportunistic gull species (yellow-legged *Larus michahellis* and lesser black-backed *Larus fuscus* gulls) from three different wildlife rescue centres that represent foraging and dwelling areas with different levels of urbanization, and from a feeding experiment of natural, urban and landfill diets. The two main goals were to 1) assess differences in gulls' diet nutritional quality among differently urbanized habitats and between different diets, through the analysis of gulls' FA composition, and to 2) investigate the possible sub-lethal impacts of ingesting debris materials, a toxicological stressor, on gulls' FA composition. Using GC-MS to identify FAs, significant differences in gulls' FA composition were detected among the three urbanization levels and between the three diets. Gulls from the most urbanized location, anthropogenically fed and landfill-caught gulls had lower percentages of physiologically important omega (ω)-3 FAs, consistent with a diet based on anthropogenic food resources. Landfill gulls had the highest ω -6: ω -3 FAs ratio, which may be associated with a diet-induced susceptibility to inflammation. We were unable to detect any effect of the ingestion of debris materials on gulls' FA composition, probably due to bias introduced in our study by using individuals from rescue centres, or due to the small amount of ingested debris materials detected, (i.e. below toxic levels), contrarily to what we expected. These data, however, constitute a valuable contribution to the limited FA literature in gulls. We encourage further studies to understand the long-term physiological impacts of a poorer nutritional quality diet for urban dwellers and to detect potential sub-lethal impacts caused by ingestion of debris materials.

2.06 Multi-stressor ecotoxicology in aquatic ecosystems in a rapidly changing climate (Poster)

2.06.P-Th037 Exposure to an Invasive Red Seaweed Exudate Under Short-Term Heatwave Events: Oxidative Stress Status and Genotoxic Effects in the Marine Mussel *Mytilus galloprovincialis*

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Climate change is leading to increased mean sea surface temperatures and extreme heat events. Temperature has a strategic influence on the physiology of poikilothermic organisms, affecting various biological processes. Thus, there is an urgent need to better understand how marine organisms respond to these rapid changes. Marine heatwaves have been attracted considerable scientific and public interest, but more knowledge is needed, especially in combination with co-occurring stressors that organisms commonly face in the marine environments. Also, invasive species are an important driver of biodiversity loss, causing declines and range shifts in other marine species. *Asparagopsis armata* is considered an invasive seaweed in the Atlantic and Mediterranean coasts. Exposure to *A. armata* exudate has been found to exert toxic effects at low concentrations to several macroinvertebrates, but knowledge on their impact under relevant scenarios including in combination with additional disturbances is still limited. Here, we focused on the responses of the mussel *Mytilus galloprovincialis* an ecologically and economically important species in coastal areas. Mussels were exposed to 0 and 2% exudate under different temperature scenarios: constant temperature (18°C) and two varying temperature conditions simulating heatwaves (increasing from 18°C to 22°C (low) and 26°C (high) for 7 days and decreasing again to 18°C). Effects of this stressor combination were assessed by evaluating DNA damage (comet assay) and biochemical alterations related to oxidative and neurophysiological stress as well as byssus production. Results

show that exposure to the exudate led to an increase in DNA damage in relation to control and a reduction in the byssus production. On the other hand, a potential reduction of neurotransmission processes in mussels' gills exposed to high heatwave scenario was observed. Mussel ability to produce byssus was impaired the most by the combined treatment of exudate and high heatwave, representing a decline of 38.6% relative to control. Furthermore, no significant oxidative stress damage caused by exposure to the *A. armata* exudate and/or by the temperature scenarios was observed in the gills, probably due to the involvement of antioxidant defences (e.g. catalase). This study brings new insights on effects of invasive species under heatwave scenarios, being such approaches crucial when determining the likely responses of marine species to environmental change.

2.06.P-Th038 Combined Effects of Boat Engine Effluent and a Simulated Heat Wave on Fertilization and Early Development of the Sand Dollar *Dendraster excentricus*

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Marine pollution and climate change concurrently affect marine environments, and both are predicted to intensify in the future. However, we lack a thorough understanding of how these events interact and may affect marine organisms in concert. A substantial fraction of coastal pollution comes from maritime activities. Recreational boat engines are known to release potentially toxic substances, such as heavy metals and polycyclic aromatic hydrocarbons (PAHs), into the water, but surprisingly little is known about the effects of boat engine effluent on marine zooplankton. Here, we tested the effects of effluent from a two-stroke gasoline outboard engine on fertilization success and early development of the sand dollar *Dendraster excentricus* at a range of dilutions. Exposure tests were conducted at ambient summer water temperature (13°C) as well as increased temperature (by 5°C), simulating a marine heat wave. Fertilization success was determined microscopically. Growth of embryos and larvae was measured for three days post fertilization and larval development was observed for signs of malformations. The engine effluent was analyzed for the composition and concentration of heavy metals and PAHs. Fertilization success varied between 77 and 92% and was affected neither by the effluent nor by increased temperature. At the end of the exposure, larvae in the heat wave treatments were consistently larger than larvae at ambient temperature. Exposure to engine effluent showed a small but significant concentration-dependent effect on body length and width at both temperature regimes (5-10% reduction compared to the control). Additionally, at ambient temperature the length of the anterolateral and post-oral arms significantly decreased with increasing effluent concentrations (by up to 27% and 16%, respectively). This was not observed in the heat wave treatment. We did not find any interaction between the two stressors. However, our observations could point towards higher temperatures mitigating some of the effluent effects on normal growth that were observed at ambient temperature, at least in the short term. Overall, our results show a potential for effluent of common boat engines to affect larval growth and development, and we will discuss the significance of these findings with respect to realistic current and future scenarios of marine pollution and climate change.

2.06.P-Th039 Potential Changes in Biofilm Functioning in Rivers Due to Vegetation Fires

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Globally, each year vegetation fires burn an area as large as the European Union (3-5 Mill. km²/year). Due to global change, ecosystems that to date had rarely experienced burning are becoming increasingly disturbed by fire. During burning, the incomplete combustion of biomass forms pyrogenic organic matter which can be transported into rivers via water-driven erosion. The effect of pyrogenic organic matter on river functioning to date remains poorly understood. In this in-situ flume study, a post-fire scenario was simulated under river-like conditions and compared to control in-situ flumes. We found that wildfire-derived pyrogenic organic matter can change biofilm functioning by modulating riverine organic matter composition and concentration, as well as pH. Furthermore, toxic substances generated and/or mobilized during the fire may also be introduced to the river and impact biofilms. These substances can include pyrogenic organic matter-bound persistent free radicals, which can generate reactive oxygen species. These in turn can cause oxidative stress to biofilms. We also observed the deposition of micron-sized charcoal particles on biofilms, which could additionally modulate their functioning. This study highlights the importance of pyrogenic organic matter for aquatic ecosystem functioning in fire-affected rivers. references:

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2.06.P-Th040 Wildfire Ashes As Risk Factor for Aquatic Invertebrates: MOLECULAR Response on *Chironomus riparius*

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Climate change causes critical alterations on world dynamics with notable increment in temperature (T) deriving in heat waves (HWs). HWs with long-term drought, are the main contributors to wildfires even aggravating their severity and frequency, particularly in Southern Europe. Wildfires have become a relevant environmental problem even on and off-site, polluting nearby water bodies. The wildfire ashes are remarkable complex matrices including heterogeneous mixture of pollutants with considerable presence of metals and polycyclic aromatic hydrocarbons (PAHs) potentially toxic to aquatic life. Diverse alterations have been observed on different freshwater trophic levels, including growth inhibition on microalgae, aquatic plants and feeding inhibition on aquatic invertebrates. However, it remains unclear which mechanisms underlie the effects of wildfire ashes at sub-organismal level. In this study, we evaluated the impact of wildfire ashes from low (LS) and high (HS) severity origin, on the *Chironomus riparius* larvae at a transcriptional level. For that, organisms were exposed to a range of concentrations of aqueous extract of ashes (AEAs) at 12.5%, 25%, 50%, 75%, and 100% for each type for 72 hours. The transcriptional response was studied

using a specific array including 42 genes related to vital metabolic pathways' through Real Time PCR (RT-PCR). The metal composition from the extracts was analyzed by coupled plasma mass spectrometry (ICP-MS). The results showed a strong disruption on transcriptional response with all the systems altered (endocrine system, detoxification, stress, immune and DNA repair) in some way more after HS ashes exposure, mainly with upregulation, worrying due to their role in vital functions. In both, LS and HS ashes, higher concentrations were detected for Mn, Zn, or Pb being the main toxicity elements. In consequence, wildfire ashes were able to modify the development of *C. riparius* as evidenced by the strong disruption on genes related to essential hormones. Moreover, the activation of crucial systems (detoxification/ stress) could limit its future response to external aggression. Finally, the DNA repair modulation emphasizes the genotoxicity of these complex mixtures. The main advantage from this study is the early detection, preventing future damage to Chironomids populations. Overall, wildfire ashes showed to be a significant environmental risk to *C. riparius* even at this short exposure time and low concentrations (12.5%). This study supports the use of transcriptional markers for an early detection of potentially toxic effects of wildfires runoff on freshwater organisms.

2.06.P-Th041 A Transportable Temperature and Heatwave Control Device (TENTACLE) for Laboratory and Field Simulations of Different Climate Change Scenarios in Aquatic Micro- and Mesocosms

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Future global climate change with higher mean temperatures and an increased severity and frequency of heatwaves as extreme weather events will affect various aquatic ecosystems to, yet, unpredictable severity and consequences. Although models suggest for series of climate change scenarios increased risk of species extinction up to the year 2050, the environmental complexity of ecosystems towards temperature changes and additional anthropogenic impacts, such as chemical release, may cause unconsidered multiple stressor interaction effects. Ongoing efforts to better comprehend such temperature-chemical interaction effects comprise almost exclusively experimental designs with a simplified approach of constant temperature regimes instead of environmentally realistic daily temperature variations. We describe here an Arduino-based temperature and heatwave control device (*TENTACLE*) that is transportable, inexpensive, multifunctional, and easily reproducible. *TENTACLE* offers water temperature monitoring and manipulation of up to 3 different climate change-related scenarios: i) current natural (ambient) sinusoidal fluctuations (suitable for indoor applications), ii) future elevated fluctuations, and iii) future heatwaves as extreme events offer significant improvements and new possibilities in experimental designs. The addition of replaceable heating element equipment and field-study suitable low-cost materials creates high flexibility for researchers who may conduct indoor or outdoor, small-scale or large-scale experiments, in fresh- or salt waters, and in different geographical locations.

2.06.P-Th042 Lumbriculus variegatus, How the Blackworm Tolerates Thermal and Toxic Stress

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The California blackworm *Lumbriculus variegatus* (*Oligochaeta*, *Annelida*) is an endobenthic sediment-ingesting worm species that presents several advantages to be used in ecotoxicology, from its relevant role on leaf decomposition, improving sediment oxygenation, with a great ability of body regeneration, which allows asexual reproduction. Still, this species is not widely used in freshwater ecotoxicological assessments, namely regarding combined stressors such as different temperatures and chemical substances in a joint exposure. To overcome this data gap, mercury (Hg) was used as a model chemical, and the standardized OECD Test Guideline 203 96 hours acute test was followed, exposing blackworms to solely-water different Hg concentrations (14, 30, 60, 120, and 240 µg Hg/L) plus an ASTM (American Society for Testing and Materials medium) negative control. Concurrently, and to evaluate how and to what extent the toxicity of Hg can change at different temperature exposures, dose response tests ran at different temperatures scenarios (10, 15, 20, 25, 30°C). Different endpoints, from subcellular to individual endpoints, were assessed in the present work. At 96h, living organisms were then divided in two groups. The first group was used to assess the blackworms' Hg content by atomic absorption spectrophotometry with thermal decomposition using an Advanced Mercury Analyser (AMA) LECO 254. The other group was used to evaluate genotoxicity by utilizing the comet assay method. Mortality was reported with LC_{50s} derived for the different temperatures (10, 15, 20, 25, 30°C) of 104, 93.3, 166, 107, 177 µg Hg/L, for each respective temperature. Bioaccumulation and genotoxicity results are still ongoing, and data will be presented at the meeting. This study aims to contribute to a more profound knowledge of the combined effects of chemicals and temperature stressors in *L. variegatus*, additionally providing some hints on the blackworm's temperature tolerance.

2.06.P-Th043 Warming Lowers Critical Thresholds for Multiple Stressor-Induced Regime Shifts Between Aquatic Primary Producers

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In shallow aquatic ecosystems, external stressors can induce regime shifts from macrophyte- to phytoplankton-dominated states

with severe consequences for ecosystem functioning and services. Shallow lakes are the most frequent freshwater type, worldwide, but effects from multiple stressors in the context of global warming are mainly undescribed. In agricultural landscapes they are exposed to mixtures of nutrients and pesticides, which can interact with other stressors as global warming in antagonistic, synergistic or reversed forms. However, to derive scientifically informed thresholds for regime shifts in the context of multiple stressors, interaction patterns need to be understood in a dose-dependent way. We tested the effects of global warming, nitrate, and a selection of pesticides typically found in agricultural run-off in a microcosm study. Effects on main autotrophic components of shallow lakes (macrophytes, periphyton, and phytoplankton) were assessed in a factorial dose-response experimental design applying stressors individually and in combination at 5 concentrations in two temperature settings. Individual stressors did not induce a regime shift, but in combination, pesticides and nitrate interacted synergistically, inducing a shift wherein phytoplankton biomass increased and macrophyte biomass decreased. Global warming amplified this effect and lowered critical thresholds for regime shifts. In conclusion, global warming increases the risk of shallow aquatic ecosystems for shifting to a turbid, phytoplankton-dominated state, and negatively impacting ecosystem service provisioning. Our study further confirmed the need of using a dose-dependent experimental design to conclude interaction patterns and critical thresholds for adverse ecological effects. Multiple stressor interactions must be considered when defining safe operating spaces for aquatic systems.

2.06.P-Th044 Can Seawater Acidification Influence the Effects of Three Antibiotics, Alone and As a Mixture, on the Early Life Stages of the Sea Urchin *Paracentrotus lividus*?

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Amoxicillin, trimethoprim and ciprofloxacin are among the best-selling antibiotics worldwide and among the most relevant in aquatic ecosystems, having an average concentration of 50-100 ng/L. As a consequence of the increased use of antibiotics, their concentration in coastal systems is also expected to increase in the years to come. In the same time frame, the increase in CO₂ absorbed by the ocean is predicted to continuously lower the pH up to -0.4 units at the end of the present century, reaching an average value of 7.7. In the present work, embryos of the sea urchin *Paracentrotus lividus* were reared in the presence of the three contaminants mentioned above (individually and in mixture) at an environmentally relevant concentration (100 ng/L) in artificial seawater at natural pH (8.1). Moreover, embryos' performances were analyzed in a multiple stressor scenario using the same contaminants but in seawater at pH 7.7. Embryos were obtained pooling gametes of 3 females and 3 males. The experiment was replicated 4 times. Embryonic development was followed for 48 hours post-fertilization by assessing the effects of the experimental conditions on developmental rate, percentage of larval abnormalities, larval growth, oxygen consumption and activity of oxidative stress (superoxide-dismutase SOD, catalase CAT), xenobiotic metabolism (glutathione-S-transferase GST) and neurotoxicity (acetylcholinesterase AChE) related enzymes. Neither the contaminants nor the pH caused a delay in the development or modification in the oxygen consumption rate. On the other hand, in larvae morphological abnormalities and skeletal rod length were significantly influenced by the pH 7.7, but not by the contaminants. Although not significantly, enzymatic activity was influenced by both contaminants and reduced pH. Biochemical biomarkers, SOD and GST activity in particular, showed a generally pejorative trend effect in larvae exposed to the mixture. These results suggest that *P. lividus* larvae are able to cope with the presence of antibiotics and reduced pH, but might face a cost at the sub-cellular level where oxidative stress and xenobiotics can cause damages. Further analysis shall be carried out to fully understand if the combined exposure to antibiotics and reduced pH jeopardize the maintenance of sea urchin populations in the future.

2.06.P-Th045 The Effects of WATER Quality on Natterjack Toad Survival and Development

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The Anthropocene has seen the global decline of amphibians with 40 % of species threatened with extinction. In the UK, amphibians are also declining, particularly the natterjack toad (*Epidalea calamita*) which is threatened with extinction in Scotland. A widely documented threat to the survival of natterjack toads is habitat changes, characterised through the reduction of grazing regimes. However, even in areas where there is suitable habitat, natterjack toads are failing to recolonise, indicating that additional threats are also impacting their survival. Here, we investigated the impacts of water quality on survival, growth, and metamorphosis of natterjacks over two breeding seasons. The basic experimental design comprised rearing spawn collected from a site with a large natterjack toad population (Cumbria, England) with water collected from prospective natterjack toad breeding pools from five sites in Scotland. In year one, for three out of the five sites tested, 100 % mortality occurred at the hatching stage. This was likely due to the high salinity recorded in these sites, due to seawater inundation (a result of climate change). For the remaining two sites, natterjack toads reared in water from Mersehead – which is the only site in Scotland with a viable population – displayed higher growth compared to Southernness, which was deemed a historical site. In year 2, survival was > 90 % for both Mersehead and the other selected sites, which had previously supported natterjack toad populations (Caerlaverock, Newfield, Moss-side). However, metamorphs reared in water collected from the historical sites were smaller than those reared in water from Mersehead. Water quality differed at Mersehead compared to the three historic sites, which included higher pH, lower conductivity, and lower total dissolved solids observed at Mersehead. These differences found potentially explain why there is an absence of natterjack toads from previously viable sites. Further work will help elucidate the potential impacts of water quality on natterjack health and survival, with the ultimate aim to prevent extinction of the species in Scotland.

2.06.P-Th046 The Acute and Chronic Effects of Chemical Exposure and Climate Change on the Life History Traits and Gene Expression of Resurrected *Daphnia magna* Clones in a Multiple-Stressor World

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The resilience of an organism is characterized by good adaptation and the resulting robustness to constantly changing environmental conditions. Due to increasing anthropogenic influences such as population growth, intensification of agriculture, advancing global industrialization and the climate change, conditions in different ecosystems are changing rapidly, making quick and targeted adaptation difficult for many organisms. However, ecotoxicology is generally concerned with the acute and chronic effects of contaminants on aquatic organisms and the environment over a short period of time, which would not consider genetic adaptations in exposed populations. Thus, long-term studies are critical to understanding ecological and evolutionary processes in nature and in assessing how species respond to and persist during environmental change. The emerging field of the evolutionary toxicology examines effects of pollutants on organisms both at the organismic and the molecular level. When combined with resurrection ecology approaches, evolutionary toxicology provides a novel opportunity to examine evolutionary adaptation at the genetic level to the deteriorating environment conditions over a long period of years to centuries without the traditional limitations of long-term or multi-generational studies. Resurrection ecology utilizes dormant eggs, for example of the keystone species *Daphnia*, retrieved from dated lake sediment cores that are hatched from over years to centuries ago. The aim of this research is to examine the effects of climate change and chemical exposure on the model keystone organism *Daphnia magna* using resurrection ecology approaches. The chemical test substance is the polycyclic aromatic hydrocarbon (PAH) phenanthrene. This is particularly suitable because it is a historical pollutant, which already occurs in the past due to incomplete combustion. Chronic (21-d reproduction test, OECD 211) and acute (Immobilisation test, OECD 202) effects on resurrected ancient and modern *D. magna* clones will be investigated using standardized bioassays to assess apical endpoints. Furthermore, qPCR is used as a molecular measurement tool to determine genetic adaptation via the measurement of gene expression of survivorship loci. The *Daphnia* clones for these studies were obtained from two lakes with different characteristics (Stadtsee, Germany & Lake Ring, Denmark). Historical data is available for both lakes. Preliminary results are already available for this study.

2.06.P-Th047 Multistressor Effects on Eukaryotic Phytoplankton. the CASE of Glyphosate and One Toxic Cyanobacterium on a Microalgal Experimental Community

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Eutrophication is a current environmental problem related to the degradation process in limnetic ecosystems; this is caused by the discharge of limiting nutrients that could favor the development of harmful cyanobacterial blooms. Also, the discharge of toxic pollutants provokes adverse effects in the phytoplankton. Chemical pollutants in eutrophic environments can stimulate cyanobacteria to synthesize and release cyanotoxins. Toxic effects of herbicides such as glyphosate-based formulations, and the presence of cyanotoxins, can act jointly to increase the stress in microalgae, contributing to the modification in the algal community. This study assessed the alteration effects of an experimental microalgal community exposed to Faena® (glyphosate) combined with one toxigenic cyanobacterium. The effects of *Microcystis aeruginosa* on population growth of the green microalgae *Ankistrodesmus falcatus*, *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, and *Scenedesmus incrassatulus* was determined. In addition, the simultaneous effects of subinhibitory concentrations of Faena® (glyphosate) on the content of macromolecules, the enzymes SOD, CAT, and GPX, as well as in the concentration of T-BARS, were evaluated. In the assays without Faena®, the microalgae showed lower growth rates than *M. aeruginosa*. In the algal community + cyanobacteria, the herbicide reduced the growth rates of microalgae, but that of *M. aeruginosa* increased. The IC₅₀'s for Faena® varied from 1,022 to 2,702 mg L⁻¹ (as glyphosate) for the different microalgae. Exposure to Faena® stimulated the cyanotoxin synthesis. The simultaneous action of both stressors produced changes in growth rates and population dynamics and increased the macromolecule content in microalgae. The increase in CAT and GPX enzymes denoted oxidative stress in phytoplankters. The integrated biomarker response (IBR) indicated that the sensitivity to stressors depended on the algal species. In the experimental algal community, the presence of *M. aeruginosa* increased the chemical stress produced by the herbicide. These results warn about the possible consequences of chemical pollutants when combined with the flourish of toxigenic cyanobacteria on the structure and dynamics of the phytoplankton community in eutrophic environments.

2.06.P-Th048 Assessment of the Toxicity of Particles and Leachate From Polyethylene Terephthalate (PET) in Combination With Triasulfuron Using Lemna minor As Freshwater Model Organism

Patricia Caballero Carretero¹, Victor Carrasco Navarro², Jussi Kukkonen² and Jose-Luis Martinez-Guitarte³, (1)National Distance Education University (UNED), Spain, (2)University of Eastern Finland, Finland, (3)UNED, Spain

Caballero-Carretero P.¹, Carrasco-Navarro, V.², Kukkonen, J. V. K.², Martínez-Guitarte JL¹.¹ Group of Biology and Environmental Toxicology, Department Mathematical and Fluid Physics, Faculty of Sciences, National Distance Education University (UNED), Madrid, Spain. ² University of Eastern Finland, Department of Environmental and Biological Sciences, Yliopistoranta 1 E, Kuopio FI-70211, Finland. The discharge and accumulation of plastic waste in worldwide waters is a disturbing situation at present. In addition to the immense number of microplastic particles present virtually in every environmental compartment, chemical additives have been also a cause of concern. Polyethylene terephthalate (PET) is one of the most popular thermoplastics used in packaging foods and beverages and textile fibers. Currently, information on the joint toxicity of PET or its additives and other chemical stressors is scarce. In our study, the toxicity of PET microparticles and leachates at three different concentrations was evaluated separately and in combination with the herbicide triasulfuron (0.1, 0.24 and 0.4 µg L⁻¹), using the common duckweed *Lemna minor* as freshwater model organism. The monitored endpoints were number of fronds, their area and dry weight. These endpoints were used to obtain growth rates and inhibition in the growth rates for each test

treatment. The preliminary results corroborated the toxicity caused by triasulfuron, but discarded any toxicity caused by PET microplastics separately or in combination with triasulfuron. The experiments with the PET leachates will elucidate whether any combination of chemical additives leaching to water cause an enhanced toxicity when combined to triasulfuron. The results of this work will provide more information about the joint toxicity of plastics, their additives and organic pollutants to freshwater organisms, helping to establish guidelines for their use and disposal.

2.06.P-Th049 Molecular Analysis of Single and Mixture Exposure of Microplastics and Thallium in the Freshwater Snail *Physella acuta*

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Caballero-Carretero P., Martínez-Guitarte JL. Grupo de Biología y Toxicología Ambiental. Facultad de Ciencias. UNED, Madrid, Spain. Nowadays, microplastics (MPs) are an increasing challenge as an environmental pollutant. MPs are present in the aquatic environment affecting organisms that live in it. They can adsorb organic or inorganic compounds acting as carriers to enter the living organisms. One of such compounds might be thallium, a highly toxic metal. It is present in the environment because of its use in industries, burning of coal, metal mining, and smelting. Moreover, it is one waste from electronic trash. It has been shown that thallium is present in aquatic ecosystems. However, only a few studies are assessing the toxicity of thallium in freshwater ecosystems, and none of them examine the effect at the molecular level. This study assesses the effect of the exposure to MPs and thallium nitrate and their mixture to analyze a putative synergy or antagonism. For this purpose, to analyze changes in gene expression, an array covering different cellular processes has been designed for *Physella acuta*. It is a freshwater snail that lives in ponds and lakes. Two-month-adult snails were exposed to different concentrations of microparticles of polystyrene (8 µm), thallium nitrate, and their mixture to evaluate the transcriptional activity by Real-Time PCR. It has been used genes related to hormonal pathways, stress response, DNA repairing mechanisms, oxidative stress, and detoxification mechanisms. We also have examined the activity of the enzymes GST, phenoloxidase, and acetylcholinesterase. The results obtained in this work provide the first approach at the molecular and cellular levels to elucidate the impact of both pollutants and their mixture in gastropods.

2.06.P-Th050 Copper Sensitivity in Three Common Arctic Copepods

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The current climate crisis increases temperature in polar regions faster and with greater magnitude than anywhere else on the planet. The decline of Arctic Sea ice extent and thickness during summer and winter opens up new passages. This development will lead to higher shipping, mining and other anthropogenic activities in Arctic regions. Intensifying anthropogenic activity will increase the risk of direct contaminant transport to the polar regions, adding to other transport pathways such as air and ocean currents from temperate regions. The shipping industry uses copper as an antifouling coating. Copper is also a trace element that is essential for many metabolic processes. However, copper becomes toxic at higher concentrations, especially for unicellular algae and invertebrates. Copepods are small but abundant crustaceans and a crucial link in the marine food web. Copper affects copepods by lowering reproductive output, prolonging developmental time, and causing mortality. The sensitivity, however, varies within and between species and also depends on the abiotic conditions. Data of copper sensitivity of polar copepods at low temperatures are rare. To fill the knowledge gap, we conducted survival experiments onboard the research vessel “Kronprins Håkon” with the three most common calanoid copepod species (*Calanus finmarchicus*, *C. glacialis*, *C. hyperboreus*) of the Arctic region. To analyse the data, we used a reduced GUTS analysis. We then placed our results in the context of published data on copper sensitivity of Antarctic and Arctic copepod species. The sensitivity of Cu exposure was comparable between the three *Calanus* species. Compared to published data from Antarctic species and tests done at low temperatures, the three *Calanus* species appeared to be less sensitive to copper. We discuss whether this is a general pattern or potentially caused by differences in life-history traits, energy reserves, body size, or other factors. Although it appears that the three Arctic species are less sensitive to copper, it has to be noted that the experimental temperature was close to the freezing point, reflecting the in-situ conditions. With rising temperatures caused by global warming, uptake and elimination kinetics are expected to become more rapid, which could well increase the copepod's sensitivity to copper, especially during short-term exposure.

2.06.P-Th051 Characterization of Three Potential Calcification Enzymes in the Mantle of Lab-Raised Pulmonate Snail (*Lymnaea stagnalis*)

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Calcifying organisms are constantly suffering from various negative impacts induced by climate change, such as CO₂-induced acidification, which may impair the formation of calcareous structures. Freshwater mollusks, likely suffer more from CO₂-induced acidification than marine calcifiers due to the lower buffering capacity of most freshwater systems. From a biochemical perspective, there are many important enzymes contributing to the biomineralization reaction. Examples include carbonic anhydrase (CA), the proton pump, and the plasma membrane calcium ATPase (PMCA). Specifically, carbonic anhydrase catalyzes the conversion from carbon dioxide to bicarbonate, as the major carbon source of the calcareous structure in calcifiers, while the proton pump acts to export the protons, generated from the calcification reaction, and thereby defend cellular pH. PMCA is proposed to import calcium from the surrounding freshwater environment, which is the major calcium source for the calcareous structure. In this study we characterized the α -CA isoform 2 (LsCA2), the proton pump subunit A (LsVHA-A), as well as partial PMCA (LsPMCA) from the freshwater snail *Lymnaea stagnalis*. We obtained the full encoding sequences, conducted

primary structure and gene expression analysis for all three enzymes, accompanied by enzyme activity measurements for carbonic anhydrase. LsCA2 is mainly expressed in the mantle tissue, which has long been proposed as the major site for biomineralization, and also expresses both LsVHA-A and LsPMCA. Further, expressions of all three enzymes are evident throughout embryonic development during which shell formation is occurring. Moreover, the primary protein structure analysis of LsCA2 and the LsCA2 enzymatic activity measurements both suggest that the LsCA2 might be embedded in the cell membrane, with the active site exposed to the extracellular environment. These findings form a strong basis for a more detailed physiological understanding of environmental effects of elevated CO₂ on calcification in freshwater mollusks.

2.07 Stress impact in food webs and across ecosystem boundaries

2.07.T-01 Ecosystem Metabolism in Streams Covering a Gradient in Agricultural Pesticide Exposure

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Many factors related to land use influence stream ecosystem functions either positively or negatively depending on stressor type and intensity, but functional redundancy may mask some or all such influences. Very few studies have focused on pesticide effects on ecosystem functions at the ecosystem scale, and no studies have addressed potential effects of pesticides on ecosystem metabolism in streams. Our objective was to explore agricultural pesticides as a potential regulating force of ecosystem metabolism in streams. We conducted comprehensive field work in 19 Danish streams covering a gradient in agricultural intensity and measured pesticide exposure, diurnal oxygen fluctuations, and physical, chemical, and biological community information (surveys of higher plants and macroinvertebrate communities) to explore drivers of ecosystem metabolism. Based on quantified pesticide exposures, predicted pesticide toxicity towards algae (sumTUalgae) was more than one order of magnitudes higher than predicted pesticide toxicity towards arthropods (sumTUdaphnia), and pesticide concentrations and TU values generally increased with increasing agricultural intensity in a two-sided 100 m buffer zone extending 2,000 m upstream of the sampling sites. Ecosystem respiration was primarily correlated to standing biomass of aquatic plants, amount of dead organic material, and macronutrient concentrations. However, primary production was most strongly correlated to sumTUdaphnia and increased significantly with increasing sumTUdaphnia, whereas sumTUalgae did provide any significant correlations to any measure of ecosystem metabolism. We propose that our results reveal cascading effects of insecticidal toxicity probably reducing standing biomass of microalgal invertebrate grazers. This leads to higher contributions of microalgal primary production influencing the metabolic balance of streams at the ecosystem level. We conclude that indirect effect mechanisms may exert as strong influence as direct effect mechanisms at the ecosystem level, and that toxicity predictions based on direct effects alone cannot reliably predict effects on ecological functions at the ecosystem scale.

2.07.T-02 Standard vs. Natural: Stressor Responses of Three Methods to Investigate Organic Matter Decomposition in Streams

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The decomposition of allochthonous organic matter is the base of the so-called brown food web, which dominates in headwater streams. Diverse agricultural stressors, such as pesticides or excessive nutrient input are known to alter decomposition by affecting aquatic hyphomycetes, the keystone fungal group for this ecosystem function. Organic matter decomposition is typically measured by deploying bags filled with leaves in streams and subsequently calculating the leaf mass loss. This, however, is labour intensive and can show high variability. Aiming to overcome this, standardised methods have been developed, but it remains unknown to which extent these respond to agricultural stressors such as pesticides. We compared two standardised methods, cotton strips and decotabs, to the traditional leaf bag method and assessed their association with extreme values of various agricultural variables in a nationwide monitoring (KgM) comprising 70 sites. Despite standardised methods being developed to decrease variability, in our study the inter-replicate variability per site of the two standardised methods was clearly higher than of the leaf bag method. Furthermore, the two standardised decomposition methods showed no or only a weak relationship to leaf decomposition, while they related stronger to each other. This lack of relationship between the leaf bag method and the two standardised methods is in accordance with previous studies. The decomposition determined with the three applied methods was explained by different variables. Leaf mass loss was explained by the ratio of agricultural land use and the maximum stream water concentrations of the trace metals arsenic and mercury. Cotton strips and decotabs decomposition were best explained by the maximum total phosphate concentration, however, exhibiting inverse relationships (Cotton strip negatively, decotabs positively related). In contrast to previous studies, none of the methods responded to the estimated pesticide toxicity towards fungi (i.e., of fungicides). The leaf bag and the standardised methods seem to capture different facets in the ecosystem function of decomposition and thus respond to different environmental drivers, which questions the use of standardised methods to estimate effects on the ecosystem function of leaf decomposition.

2.07.T-03 Aquatic-Terrestrial Linkages: Cascading Contaminant- or Emerging Insect-Mediated Effects on Riparian Spiders

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Organic pesticides contaminating freshwater ecosystems, have the potential to change aquatic insect development and emergence. Additionally, emerging aquatic insects are known to transport a variety of anthropogenic micropollutants from aquatic to terrestrial ecosystems. Exposure of aquatic insect larvae to pesticides can therefore impact terrestrial food webs by direct dietary pesticide exposure or changes in the quantity or timing of emerging insect subsidies. Knowledge on the effects of pesticide- and organism-specific parameters, such as pesticide class and insect sex-specific life history, as well as concentrations of pesticides in exposed riparian spiders is lacking for many current-use pesticides in this context. We conducted laboratory microcosm scale experiments simulating two field relevant exposure scenarios, i.e. acute pulse exposure to single insecticides and chronic exposure to a mixture of fungicides and herbicides during the aquatic development stage of the midge, *Chironomus riparius*. We measured pesticide concentrations in the developing larvae, directly after emergence and at the end of the terrestrial life stage of the adults. Additionally, we measured effects on larval development time, emergence success and emergent biomass. Furthermore, field sampling of riparian spiders and adjacent aquatic ecosystems from river sites along a gradient of agricultural land use was conducted. These samples were analysed for a broad range of current-use pesticides and organic micropollutants. Exposure to pesticides during the larval development of midges resulted in potential negative effects for terrestrial consumers regardless of the exposure scenario, pesticide class or insect sex. Acute exposure to sublethal concentrations of insecticides affected development duration and emergence success. Chronic exposure to a mixture of fungicides and herbicides resulted in the majority of pesticides being retained in the adult insects after emergence. Furthermore, sex-specific effects were observed in larval development duration and pesticide concentrations in the emergent adults. Pesticides concentrations measured over the terrestrial life stage also exhibited sex-specific changes. A broad range of contaminants were measured in the field-collected riparian spiders. Pesticide concentrations in spiders were found to correlate with sampling site contamination and revealed potential biomagnification through the consumption of aquatic prey for some contaminants.

2.07.T-04 Carry-Over and Cumulative Effects of Opioid Exposure in European Common Frogs (*Rana temporaria*)

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Early-life exposure to environmental stressors can affect individuals later in ontogeny, particularly in species with multiple life history stages. Many species carry over stress or information gained in these early life history stages regarding predators, climate, and contaminants, which can influence adult behavior and development. Depending on the timing of these latent responses, carry-over effects can compound with direct stressors, both natural and anthropogenic, in additive or synergistic ways. We found that larval European Common frogs (*Rana temporaria*) exposed to tramadol, an opioid contaminant in freshwater systems, accumulated the drug in their tissues at ratios consistent with exposure concentrations. We found no treatment differences in larval development rates, larval behavior, metamorphic size or weight, or metamorphic timing. Carry-effects were only detected when individuals exposed to high levels of tramadol as larvae experienced highly competitive environments as a metamorph. Competition levels were consistent across treatments during the larval stages, with larvae held in groups of 5 individuals. These groups were maintained over ontogeny as metamorphosed individuals were transferred to terrariums. As such, metamorphic competition depended on the order of emergence within groups. Early emerging metamorphs experienced little competition in their terrariums while late emerging individuals faced a terrestrial environment with four conspecifics. This priority effect was found to compound with tramadol exposure rates in interesting ways. We assayed 2-wk old metamorphs for behavioral response to novel objects using a flight initiation test. We found late-emerging individuals exhibited significantly slower motor response to an approaching novel object. This delay in response, however, was only found in individuals exposed to high levels of tramadol during the larval life stage. Carry-over effects of tramadol in this multiple stressor framework highlights the need for ecology-based contaminant studies over an individual's lifetime and in naturally relevant scenarios.

2.07.T-05 Mosquito Control Agent *Bacillus Thuringiensis* Var. *israelensis* Alters Emergence Dynamics of Merolimnic Insects From Floodplain Mesocosms

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The bacterium *Bacillus thuringiensis* var. *israelensis* (Bti) is widely used as a mosquito control agent assuming a taxa-specific toxic mode of action against larvae. However, studies investigating (sub-)lethal effects on non-target organisms, especially on non-biting midges (Chironomidae), raised concerns about the selectivity of Bti. As Chironomidae are an important link between freshwater and adjacent riparian food webs, changes in the population development of this and other aquatic insect species point to adverse implications on subsidised food webs. To address this aspect experimentally, we investigated the emergence of insects in Bti-treated and control pond mesocosms (21 m x 5.80 m, n = 6). During April and May 2020, Bti, i.e. Vectobac WDG, was applied three times at the highest recommended field rate in Germany ($2.88 \cdot 10^9$ ITU/ha) aligned with an artificially generated flooding to mimic the common application scenario of Bti. The emergence of insects in each pond was collected by six floating traps (0.33 m²) which were emptied once or twice a week from mid-April until the end of July. Emerged insects were identified to

family level and their abundance and biomass were determined. More than 30 insect families were recorded with Chironomidae being the most abundant family covering 88% of the emerged individuals followed by Baetidae constituting 9%. Investigating family-specific emergence dynamics revealed a statistically significant effect of Bti over time on Chironomidae but not on Baetidae. The emergence peak of Chironomidae in Bti-treated ponds was earlier and reduced compared to the control ponds. It is assumed that Bti induced an accelerated development of current late-stage larvae as stress-response while early-stage larvae died from Bti resulting in a decreased emergence weeks after the last application. No statistically significant effect of Bti on the mean weight per individual emerged Chironomidae was found. Nevertheless, our results point to a negative impact of Bti on non-target Chironomidae in accordance with several other studies. Additionally, an earlier peak of the key emergence (i.e., Chironomidae) may have a strong impact on higher trophic levels in terrestrial systems such as birds or spiders depending on prey availability during their reproductive phase. Furthermore, the nutritional value of the available prey should not be ignored thus we aim to quantify the energetic budget of Chironomidae from treated and untreated ponds.

2.07 Stress impact in food webs and across ecosystem boundaries (Poster)

2.07.P-We038 Direct and Indirect Effects of the Fungicide Fluopyram on an Aquatic Decomposer-Detritivore System

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Leaf litter breakdown is a key ecosystem process for energy provisioning in headwater streams and is primarily driven by microbial decomposers and detritivorous macroinvertebrates. Anthropogenic stressors like fungicides, however, can disrupt the functioning of decomposer-detritivore systems via direct (waterborne) and indirect (diet-related) pathways. Therefore, we performed a set of laboratory studies that assessed how the fungicide fluopyram affects the detritivore *Gammarus fossarum* and leaf litter-associated microbial decomposers. In the first experiment, *G. fossarum* was exposed to fluopyram (0 to 60 mg/L) over the water phase while feeding on microbially colonized leaf discs. *Gammarus*' feeding rate was assessed after seven days of exposure. Results showed that fluopyram induced 50% reduction in feeding at 1.49 ± 0.26 mg fluopyram/L. These results were used for a second experiment, during which *G. fossarum* was given the choice to feed on leaf discs for 24 hours that had either been microbially colonized in the presence (15 to 15,000 µg/L) or the absence (fungicide-free control) of fluopyram for 14 days (=diet-related pathway). The feeding preference of *G. fossarum* was estimated using its consumption on the two offered leaf discs as a proxy. In addition, the community composition, species richness, and number of released conidia of aquatic hyphomycetes (AH; a polyphyletic group of aquatic fungi) associated with the leaf discs were assessed. Fluopyram reduced the number of released conidia (~43-73%) and fungal species richness (~77-98%), while the AH community composition was concomitantly altered. These shifts at the structural level presumably affected the palatability and nutritional quality of leaf discs for *G. fossarum*, which was reflected in up to ~50% lower consumption of the fungicide-exposed leaf discs compared to the control leaf discs. This demonstrates that fluopyram likely does not pose a direct risk to *G. fossarum* over the water phase at environmentally relevant concentrations in the lower µg/L range. However, fluopyram effects on AH propagated to *G. fossarum* even at low concentrations by impacting their food choice behavior. The pronounced effect on AH pinpoints their vulnerability towards fungicides and calls for a more comprehensive understanding of each pathway's relevance to accurately predict effects on energy provisioning in heterotrophic stream food webs.

2.07.P-We039 Effects of Zinc Oxide Nanoparticles and Salinity on the Decomposition of Leaf Litter in Mangrove Ecosystems

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Mangroves exist at the boundary of terrestrial and marine ecosystems. As they are conduits for water flowing from the surrounding catchment they are particularly vulnerable to pollutants. The decomposition of leaf litter is a vital ecological process in mangrove ecosystems. The dominant mangrove species in Oman is *Avicennia marina*, which can thrive in a highly arid and saline environment. The decomposition of *A. marina* leaves drives the detrital ecosystem in mangroves. Any human activities that disrupt this process may have harmful impacts on the overall health of the mangrove system. Zinc oxide nanoparticles (nZn) are an emerging pollutant that are increasingly used in industrial applications and personal care products such as sunscreens. Toxicity is directly related to the size and physical form of the nanoparticles, which in turn depends on environmental conditions. Salinity can have a major impact on the structure and toxicity of nanoparticles but has rarely been investigated as an independent factor. The aim of this study was to investigate the effects of nZn and salinity on the decomposition of *A. marina* leaves. Leaves were collected from the Al Qurum nature reserve in Muscat, Oman. Experiments were conducted in 3L glass microcosms containing sediment from the collection site. Leaves were exposed to 1, 10, 50 mg/L nZn, or 10 mg/L ZnCl₂ in either 50 or 100% seawater for six weeks. Nanoparticle size was measured using TEM. At 2, 4, and 6 weeks leaf subsamples were collected and the decomposition rate calculated. We also measured dissolved ion zinc and fungal biomass. Effects of nZn on leaf morphology was assessed using SEM. In a second experiment we measured the effects of the nZn on decomposition in 25, 50, 75 or 100% seawater. Decomposition was inhibited by 10 and 50 mg/L nZn and was higher at 100% salinity, although there was no interaction between the factors. Fungal biomass was higher in the nanoparticle treatments compared with controls suggesting the effects were not mediated through fungal toxicity. In the second experiment decomposition rates increased with increasing salinity but were not affected by the nanoparticles. In conclusion, this study showed that salinity plays an important role in the decomposition of mangrove leaves but that higher concentrations of nanoparticles can inhibit decomposition. Future studies should examine the effects of nanoparticles on other components in the ecosystem and their interactions.

2.07.P-We040 Diuron Indirectly Affects the Feeding Activity and Physiology of the Grazer *Physella acuta* Through Shifts in the Periphyton Community Composition

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Herbicide effects on freshwater periphyton communities are well documented. Considerably less knowledge, however, has been gained for subsequent diet-related effects on primary consumers through shifts in the periphyton community composition that potentially alter the food quality for higher trophic levels. We, therefore, offered periphyton grown for 15 days in absence or presence of 8 µg diuron/L as food to the gastropod grazer *Physella acuta* for 21 days to quantify changes in its feeding rate, growth rate and energy reserves (estimated as neutral lipid fatty acids; NLFAs). In order to support a mechanistic interpretation of the grazers' responses, we quantified periphyton biomass, cell viability, community structure of autotrophic microorganisms (phenotype-based algae community composition and the diatom community) and fatty acids (FA) as a proxy for the food quality. The focus on diatoms was driven by their high content in highly unsaturated fatty acids (HUFA). Although diuron did not alter periphyton biomass or the viability of algae, shifts in the periphyton community were indicated by significant changes in the composition of algal phenotypes as well as the diatom community composition that were characterized by a shift in the relative abundance of diatoms. This shift in the periphyton community was reflected in changes of the FA profile. Especially the higher share of HUFA mirrors the increase in the diatom abundance, which may lead to a higher food quality of periphyton for grazers. The increased quality of diuron-exposed food could explain the observed stimulated feeding activity of snails in the treatment compared to the control. Moreover, the enhanced feeding activity resulted in an increase in the snails' growth rate (55%) and energy reserves (increase of NLFAs by 65%). Furthermore, snails feeding on diuron-exposed periphyton showed shifts in their NLFAs profile that pointed towards a higher physiological fitness compared to the control at the end of the bioassay. Our results indicate that herbicides can indirectly alter the behavior and physiology of grazers. If merolimnic aquatic organisms are affected, which are considered a high-quality food source for terrestrial consumers in riparian ecosystems, knock-on effects in aquatic-terrestrial meta-ecosystems are conceivable. These concerns call for a deeper understanding of herbicide effects on periphyton-grazer systems that potentially trigger consequences at the ecosystem level.

2.07.P-We041 Specific Mosquito Control Agent? Effects of *Bacillus Thuringiensis* Var. *Israelensis* on Benthos Community Structure and Ecosystem Function in Semi-Field Mesocosms

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Bacillus thuringiensis var. *israelensis* (Bti) is a widely used biocide to control mosquito populations in wetlands. Despite being considered to specifically act on larvae of target organisms, i.e. Culicidae, Bti was shown to reduce density of non-target Chironomidae in laboratory and field studies. Since Chironomidae larvae are a key food source in aquatic food webs, it is hypothesized that a possible reduction of Chironomidae larvae by Bti may also cascade on the aquatic food-web altering structure and composition of benthic communities, particularly in small lentic water bodies where many predators depend on high densities of Chironomidae larvae. Alterations in benthos community may also lead to impaired ecosystem functions such as leaf decomposition. In this experiment, we investigated changes in the benthos community structure and composition in twelve floodplain mesocosms (ponds; 21 x 5.8 x 0.3 m), from which six were treated with the maximum field rate of VectoBac WDG (i.e., three applications in six weeks), while the other six remained untreated and were used as a control. Sampling was conducted three weeks after the last Bti application in macrophytes and gravel substrate to investigate the influence of Bti on the benthos community structure, dependent on the habitat type. Organisms were identified to species (Ephemeroptera, Odonata) or family level (Diptera, Coleoptera, Mollusca). To investigate decomposition by benthic shredders, ten leaf bags were introduced to each pond after the last Bti application. Leaf decomposition was determined after 14 days and 28 days. Regardless of the treatment, habitat type 'macrophytes' showed a higher species diversity as well as a 73-fold increase in the total number of sampled individuals compared to habitat type 'gravel' ($p < 0.01$). In macrophytes, Chironomidae larvae were the most affected taxa with a significant ($p < 0.04$) reduction of ~45%. Furthermore, in ponds exposed to Bti, our results (although not significant) suggest a reduction of ~35% in the total number of dragonfly larvae (Odonata) collected in macrophytes with early instars of the family Libellulidae and suborder Zygoptera being most affected (reduction up to 45%). Such a reduction of Odonata larvae may be the consequence of a cascading effect along the food chain either due to the substantial reduction of Chironomidae abundance and/or possible cannibalism occurring in older larval stages of Odonata. We conclude that Bti can reduce the number of Chironomidae larvae and that the effects can cascade to higher trophic levels of the benthic community.

2.07.P-We042 Tropic Transfer of Pharmaceuticals in the Benthic Food Web - a Conceptual Approach

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Due to an increased and continuous human consumption and inefficient removal by wastewater treatment plants, pharmaceutical compounds are discharged continuously to the aquatic environment. Consequently, pharmaceuticals have been detected in both surface waters, biota such as fish and to some extent in sediments as it is more rarely included when doing environmental monitoring. However, to what degree most pharmaceuticals bioaccumulate through the benthic food web have to our knowledge yet to be investigated. As pharmaceutical compounds are a broad term describing diverse groups of compounds varying in chemical

properties (i.e., hydrophobicity and ionization potential) and mode of action, these compounds will distribute differently in the aquatic environment. Some of these pharmaceuticals (e.g. sertraline, citalopram, and fluoxetine) are likely to accumulate in the sediment compartment to concentrations that may exceed the concentrations of surrounding surface water. Sediment-associated pharmaceuticals may be taken up and potentially bioaccumulate in sediment-dwelling invertebrates and may further bioaccumulate in fish feeding on such prey. The aim of the study is to investigate to what degree selected pharmaceutical compounds transfer from surface water to sediment; from sediment to benthic invertebrates; and from invertebrates to fish by examining each transfer step in its own isolated system. The experiments will be conducted in the following matrices: distribution between water and sediment, bioaccumulation from sediment to benthic invertebrates (i.e., worms or sediment living amphipods) examining the whole body burden of the invertebrates and bioaccumulation from benthic invertebrates to fish by analysing target organs (i.e., liver, intestines and brain), each matrix based on the finding from the previous. The selection of pharmaceutical compounds will be based on environmental concentrations in water and sediment (if available) within the therapeutic groups of i.e., psychoactive pharmaceutical, anti-inflammatory drugs, cardiovascular drugs, antibiotics and antihistamines.

2.07.P-We043 Stable Isotope Analysis As a Promising Tool to Unveil Toxicant Effects in Food Webs

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Despite having comparable effect sizes as direct effects, indirect effects that are conveyed via food webs have received far less attention mainly due to methodological limitations. Although stable isotope analysis (SIA) is used for decades to address ecological research questions, its use in a chemical stress context has been limited. The applicability of SIA to track indirect effects in food webs has recently been challenged by studies stating an effect of chemical stressors on an organism's isotope signature. To evaluate the application of SIA, this study investigates if trophic enrichment factors (TEFs, i.e., the difference of stable isotope signatures between consumer and prey) are affected by toxicants and if estimating dietary proportions with SIA fits the observations based on consumption data. To address the former, the amphipod *Gammarus fossarum* (Koch) was subjected to three levels (i.e., 0, 0.75, 5 $\mu\text{g L}^{-1}$) of the neonicotinoid thiacloprid and fed with either black alder leaves or *Baetis rhodani* larvae over six weeks. To address the latter, gammarids were subjected to two levels of thiacloprid (i.e., 0, 0.75 $\mu\text{g L}^{-1}$) and fed with either black alder leaves, *B. rhodani* larvae or both over two weeks. The first experiment showed that thiacloprid exposure induces changes in TEFs that are statistically significant but small compared to other factors (e.g., resource quality and physiological condition) and thus likely of minor importance. The second experiment showed that dietary proportions as suggested by SIA reasonably fit those derived from consumption data suggesting its applicability. However, the experimental assessment of TEFs and a sufficient separation of resources' stable isotope signatures are principal to generate accurate and precise predictions, respectively. Labelling resources with heavy isotopes addresses both issues in one sweep, potentially even enabling SIA to track indirect effects in more complex scenarios.

2.07.P-We044 Fatty Acid Profiles of *Chironomus riparius*: Effects of Developmental Stage and Heavy Metal Exposure

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Emerging merolimnic insects (i.e., insects with an aquatic larval and terrestrial adult life stage) are important vectors for dietary nutrients such as long-chain polyunsaturated fatty acids (LC-PUFA) for riparian consumers. This is because LC-PUFA are required to maintain vital physiological processes, but, at the same time, are lacking in terrestrial prey. Anthropogenic stressors can impair the development and emergence of merolimnic insects and thus the transport of these nutrients. In this context, heavy metals and water temperature increases might be of particular concern, since they can accumulate in sediments and have a direct effect on the FA composition of aquatic organisms, respectively. Therefore, we examined the interacting effects of sediments contaminated with non-essential (cadmium, Cd) or essential (copper, Cu) metals and short-term, recurrent water temperature increases on the FA profile of different developmental stages of *Chironomus riparius* (Diptera: Chironomidae). Preliminary results of both experiments suggest that the developmental stage (i.e., Larvae, Female or Male) had a statistically significant effect on the FA composition of *C. riparius*, explaining 22% (Cd experiment, PERMANOVA, $p = 0.0001$) and 43% (Cu experiment, PERMANOVA, $p = 0.0001$) of the total variance. Nonmetric multidimensional scaling revealed that PUFA and omega-6 FA were mainly associated with males and saturated FA and total FA contents mainly with females. In addition, we observed a trend towards a higher FA contents in adults compared to larvae in the Cu experiment. Furthermore, Cd had a statistically significant effect (PERMANOVA, $p = 0.0019$) on the FA composition of *C. riparius*, with increases in omega-6 FA, PUFA and omega-3 FA along the Cd gradient being the strongest contributors to differences in FA composition (according to SIMPER analyses). Recurrent temperature increases did not significantly affect FA composition, presumably due to the short (i.e., two to three days) periods. Given that more contaminants become ubiquitous in the environment and PUFA are required for many physiological processes and are mostly synthesized in the aquatic environment (particularly LC-PUFA), contaminant-induced effects on the emergence pattern and FA profile of merolimnic insects may cause alterations in the transport of FA that could cascade onto higher trophic levels.

2.07.P-We045 Emerging Aquatic Insects Transfer Environmental Stress Across Ecosystems

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Nowadays, freshwater ecosystems are contaminated with various pollutants, which can co-occur and interact in many ways, cross

ecosystem boundaries and propagate to adjacent terrestrial habitats. Moreover, these ecosystems are subjected to climate change which can impair their normal functions and balance, e.g. changing water temperature. Emerging aquatic insects play a crucial role in these environments, transferring resources and energy, as well as subsidies to higher trophic levels in both aquatic and terrestrial habitats. Accordingly, the aim of the current study was to investigate the single and combined effects of emerging contaminants (ECs); pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) and elevated water temperature on aquatic insects at the aquatic-terrestrial habitat linkage. A laboratory microcosm experiment was conducted with a simplified freshwater food web containing nonvascular macrophytes and *Trichoptera* larvae feeding mainly as shredders. Sampling included initial and several consecutive collections including all life stages (larvae, pupae and adult stage). Analyses such as total protein content, total lipid content, metabolite profiling and fatty acids composition are being conducted in order to evaluate the response of non-model aquatic insects to selected stressors at molecular level. Increased water temperature had an impact on phenology causing earlier emergence of adults and a decrease in lipid mass. Furthermore, treatments with ECs mixture lowered the protein mass in larvae and pupae, irrespective of the water temperature. Multiple stressor effect was noticed in treatments with combined ECs and elevated water temperature as the highest decrease in total protein content in larvae and pupae and total lipid content in adults.

2.07.P-We046 Prescribed Burning As an Ecological Stressor and Its Effect on the Soil Ecosystem Services of a Fescue Prairie Grassland

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Prescribed burning is a traditional management practice used across temperate grasslands to control natural fire regimes. In the Canadian Prairies, prescribed burning is a standard management technique to maintain the productivity of native plant species. Despite the benefits of this management practice, studies have shown its effect on soil ecological functions. However, most of these studies on the impact of prescribed burning in temperate grasslands focus on the dynamics in carbon storage for pre/post-burning regimes. Therefore, in this study, we decided to investigate the effect of prescribed burning on important soil ecosystem services after four years post-burning in a fescue prairie grassland of Saskatchewan, Canada. We measured and collected 2-years field data (2020/2021) for some soil ecosystem services proxies. Briefly, we measured greenhouse gases (GHGs) as the proxy for climate regulation, tea bag index (TBI) as the proxy for decomposition, vegetation cover/biomass as the proxy for food production/productivity, and soil invertebrate population as the proxy for soil biodiversity conservation. Interestingly, the results from this study showed that prescribed burning significantly affect soil invertebrate population, especially collembola after 4-5 years post-burning. There was also a reduction in the vegetation cover/biomass in the burned plots. In addition, the collembola population was driven by changes in litter cover for the burned and unburned fields. In conclusion, prescribed burning affects the soil's ecological functions by significantly affecting litter-dwelling soil invertebrates such as collembola. Therefore, soil invertebrates population should be monitored post-burning to maintain grassland's ecosystem integrity.

2.07.P-We047 Adverse Multigenerational Effects of a Nano-Façade Paint on *Daphnia magna*

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Façade paints often contain organic biocides to protect wall surfaces from algal and fungal growth. Recent developments in related industry sectors focused on reducing the content of organic biocides, for instance by replacing them with inorganic nanomaterials (NM) that exhibit more natural biocidal properties. However, both organic biocides and inorganic NM can be washed off façades during rainfall and subsequently enter the aquatic environment. Once entered, adverse long term effects on respective ecosystems and their functions may be a consequence, with rather limited knowledge on NM effects. Thus, the present study aimed at investigating biological effects of silver NM (=AgNM) and a façade paint (=FP; containing AgNM) on the aquatic invertebrate *Daphnia magna* during a multigenerational test with three subsequent generations tested for 21d each, largely following OECD 211. Juveniles of the 3rd brood (of each treatment) from the 1st and 2nd generation served as a starting point for the following generation. The exposure of daphnids took place via algal food. In detail, 3 d before feeding, the food source was separately spiked with four different concentrations (< 72h EC₂₀ algae growth) of the two test items (AgNM [0.00, 0.08, 0.10, 0.12 mg/L], FP [0.00, 1.00, 3.00, 5.00 mg/L]). Afterwards, the spiked suspensions were used to feed daphnids (n=15). Daphnias' reproduction, growth and mortality were assessed over the entire exposure time. Results showed that even the lowest concentration affected the reproduction (~75% reduction compared to the control) of daphnids in all generations, independent of the substance tested. Similar patterns were observed for the growth of the adult daphnids. While respective effect sizes did not significantly differ over the three generations for both products (neglecting any transgenerational effects), mortality deviated significantly among generations when exposed to algae treated by FP. There, the survival of the test animals decreased significantly with increasing test item concentration and consecutive generation. For example the 3rd generation of daphnids exposed to FP showed only ~33% survival rates in the highest treatment versus ~ 100% in the 1st generation. Our findings suggest that the transport of nanomaterials along the aquatic food chain alone and as part of complex product mixtures such as façade paints may adversely affect such ecosystems, while the concentrations assessed are beyond field relevance.

2.07.P-We049 Simple Food Web Model for the Chronic Impact of Rare Earth Elements in an Aquatic Ecosystem

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The rare earth elements (REE) are 17 chemical elements that possess unique properties. These make them crucial in modern technologies such as GPS, electrical cars and agriculture. REE are considered as emergent contaminants and there is a concern about their environmental safety. It is known that REE can cause disruptions in the biogeochemical cycles of aquatic ecosystems. Benthic organisms can be exposed to these contaminants, due to the strong adsorption of REE to sediment particles and fine organic particle matter. REE can also affect species in the overlying water body due to the coupling between the benthic habitat and the pelagic zone. We have developed a simple food web model for the chronic impact of two REEs lanthanum (La) and gadolinium (Gd) containing a sediment-water interface. For this purpose, five different organisms from various trophic levels (e.g. bacteria, microalgae, nematodes, daphnia, ostracods, and mussels) will be exposed in acute and chronic environmentally relevant REE concentrations in controlled micro- and mesocosm experiments. Our prototype food web model uses the Lotka-Volterra equations and first-order linear processes for the transfer of REE in the aquatic food web. Element uptake, toxicity data and a mechanism representing the repair of damage are integrated into a system of logistic mathematical equations, set and solved in the user-friendly ModelMaker software. This is an “open model” whereby partial parameter information can be readily incorporated as information is gradually gained from the micro- and mesocosm experiments, thus refining the model parameterisation. The model developed can be employed to optimise the experimental design of the micro- and mesocosms. The key challenge in this heuristic approach to modelling is that experiments are required to recalculate the relevant rate constants and the representation of the chemistry of the REE. We further elaborate on how we are dealing with the heuristic modelling challenge in this study and the first prototype of the theoretical model is presented. We will discuss the “lessons learned” regard to the potential of this approach and considering implications of REE-effects on biological communities.

2.08 Theoretical concepts to advance prediction in ecotoxicology and stress ecology (Poster Corner)

2.08.PC-Tu19 Three Perspectives on the Prediction of Chemical Effects in Ecosystems

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A wide range of chemicals of urban, agricultural and industrial origin, including pesticides, metals, pharmaceuticals and industrial chemicals, can be detected in the environment, in particular in aquatic ecosystems, given that they often drain landscapes with diverse land use. The use and emissions of chemicals into the environment have increased at a higher rate than most other drivers of global change. Yet, the large number and increasing production volumes of synthetic chemicals, their interaction with other moderating factors and limited knowledge on exposure patterns and effects in organisms limit our ability to predict effects in real-world ecosystems. In our contribution, we identify three approaches for the prediction of chemical effects: 1) the mechanistic perspective, 2) the toxicokinetic-toxicodynamic perspective and 3) the eco-evolutionary perspective, which are currently largely separated. We describe these three perspectives, highlight methodical and conceptual advances as well as challenges and synthesise novel developments that can create links between the perspectives. Moreover, we provide guidance on how to approach the task of prediction using current tools and discuss future experiments and models that would foster our capacity to predict ecological responses in complex ecosystems. Overall, we provide a timely overview of the approaches and challenges to prediction of effects for an important global change driver that is characterised by a higher complexity than other drivers.

2.08.PC-Tu20 Comparing Recovery-Related Trait Profiles Between Sensitive and Tolerant Freshwater Macroinvertebrate Assemblages to Insecticides

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The vulnerability of assemblages to stress is a function both of their ability to resist the stressor (i.e., sensitivity) and their ability to recover from any stressor-induced impacts once the stressors have been removed. The sensitivity of an assemblage is determined by the sensitivities of the individual populations making up the assemblage and will therefore depend on species compositions. As interspecific variation in chemical sensitivity is chemical dependent, assemblages that are sensitive to some toxicants may be relevantly tolerant to other toxicants. The recovery of assemblages is a function of internal recovery processes that depend on species-specific traits (e.g., generation time, number of offspring) and external recovery processes that are a function of both species-specific traits (e.g. dispersal, life-cycle) and landscape factors (e.g., source populations, connectivity). This study aimed to investigate: (1) whether sensitive and tolerant freshwater macroinvertebrate assemblages share similar recovery-related trait profiles; (2) if they are different, what recovery-related traits contribute to this variation. Freshwater invertebrate assemblages in England were used as a case study and seven insecticides were selected as study chemicals. As the sensitivity of most invertebrates in assemblages is unknown, the hierarchical species sensitivity distribution model was used to predict the toxicity of untested species. The concentration hazardous to 5% of species (i.e., HC5) was used as a measure of assemblage sensitivity. Chemical-specific HC5 values were calculated for all assemblages and for each insecticide, sensitive assemblages were defined as those in the lowest 5% of HC5 values whereas tolerant assemblages were defined as those in the highest 5% of HC5 values. The unique taxa from sensitive and tolerant groups were identified and their recovery-related traits were determined and compared. Sensitive and tolerant freshwater macroinvertebrate assemblages exhibited different recovery-related trait profiles. Locomotion and substrate relation and dispersal mode are the largest contributors to the difference in recovery profiles. Most taxa presented active aerial dispersal behaviors in tolerant assemblages, while taxa in sensitive

assemblages exhibited diverse dispersal modes. The difference in recovery profiles indicates that external recovery may be important for assessing the ecological risk of chemicals with intermittent exposure patterns.

2.08.PC-Tu21 Predicting Effects During Stressor Impact and Beyond: Understanding the Mechanisms Governing Community Response During Stressor Action and Release With the Asymmetric Response Concept

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Predicting the effects of multiple stressors on communities is complicated by interacting ecological mechanisms that vary in importance during the time course of stressor action and release. The Asymmetric Response Concept (ARC) provides a conceptual framework to structure hypotheses and contextualize the importance of the three mechanisms, namely individual tolerance, dispersal, and biotic interactions of taxa, during the stressor impact and recovery period. According to the ARC, tolerance of individual taxa plays the most significant role as stressor impact increases. Individual tolerances of taxa, for which metrics are available for a range of stressors and organisms, are thus hypothesized to explain community response during stressor increase. Dispersal and biotic interactions become dominant after stressor release as the community recovers. Knowledge of each process can subsequently be used to predict effects at the relevant stage of stressor impact, with the goal of maximizing recovery of degraded ecosystems. A wide variety of data for single taxa tolerance to a range of environmental and chemical stressors is available in databases, for example EC/LCx values for chemicals or critical maximum values for temperature. Harnessing this information to extrapolate effects to higher levels of biological organization is of great interest to ecotoxicologists, ecologists, and risk assessors alike. Within the ARC framework, the success or failure of individual tolerance predictions on community response at different phases of stressor impact can be explained by the shifting relevance of the three mechanisms mentioned above. Illuminating which mechanism - tolerance, dispersal, or biotic interactions- governs the community response in which phase will ultimately improve our predictive capacities for effects of multiple stressors over time.

2.08.PC-Tu22 Caenorhabditis elegans Gut Microbes Decrease Arsenite and Perfluorosulfonate Toxic Effects on Nematode Reproduction and Fat Metabolism

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Environmental toxins such as arsenite (As^{III}) and Perfluorooctanesulfonic acid (PFOS) are associated with contamination of drinking water and food. Microbes that colonize the gut play an important role in host response to ingested environmental toxins. Exposure to arsenite or PFOS in animal models has shown to affect reproduction and fat metabolism, however, the effects of gut microbes on host physiological responses to environmental toxins is unclear. In toxicology, *Caenorhabditis elegans* is an invaluable model organism that shows good correlation of toxic responses with mammalian models. The aim of the study was to investigate the effect of 3-bacteria gut microbe combinations on *C. elegans* reproduction and fat metabolism in response to toxin exposure. *C. elegans* fed mixtures of 3-bacteria combinations or *Escherichia coli* OP50 (control) were exposed to As^{III} (150 µM) or PFOS (100 µM) and egg laying and the expression of genes associated with fat metabolism were analyzed. In comparison to the *E. coli* OP50 fed *C. elegans*, worms fed with the 3-bacteria models improved the nematode egg laying in the presence of either As^{III} or PFOS. Furthermore, worms fed with the above 3-bacteria combinations showed increased expression of fat metabolism genes (*elo-2*, *sams-1*), indicating gut microbes modulate the host fat metabolism and reproduction when exposed to toxins. Understanding the influence of gut microbes on host during toxin exposure provides insight into the possible mechanisms of host-microbe interactions and identifies novel biomarkers of toxicity for better prediction of the toxicological outcome.

Track 3: Environmental chemistry and exposure assessment: analysis, monitoring, fate and modeling

3.01 Advances in Bioaccumulation Science and Assessment (Part I)

3.01.T-01 Comprehensive Screening and Trophic Transfer Behaviour of Emerging Organic Contaminants in a Freshwater Food Web

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Bioaccumulation and trophic transfer of persistent legacy contaminants have been intensively characterized, but little is known on the contaminants of emerging concern (CECs) in the freshwater food web. Herein, we comprehensively screened CECs with a focus on (semi) polar substances and further evaluated their trophic transfer behavior in one plankton, two mussel, and nine fish samples of a food chain in Lake Templin from Germany. With an effective multi-residue sample preparation method and high-resolution mass spectrometry-based target, suspect, and non-target screening, we characterized 477 targets and further screened unknown features in complex biota matrices. Of the 477 targets, 145 were detected and quantified in different species (0.02 - 3640 ng/g, dry weight). Additionally, the suspect and non-target analysis with experimental mass spectra libraries and *in silico* techniques (MetFrag and SIRIUS4/CSI:FingerID) enabled further identification of 27 unknown compounds with 19 confirmed by

reference standards. Overall, the detected compounds belong to a diverse group of chemicals, including 71 pharmaceuticals, 27 metabolites, 26 pesticides, 16 per- and polyfluoroalkyl substances (PFASs), 11 industrial chemicals, 4 plasticizers, 3 flame retardants, and 14 others. Moreover, we determined the trophic magnification factor (TMF) of 34 CECs with >80% detection frequency, among which 6 PFASs including perfluorooctanesulfonic acid (PFOS), perfluorodecanoic acid (PFDA), perfluorohexanesulfonic acid (PFHxS), perfluorotridecanoic acid (PFTrA), perfluorotetradecanoic acid (PFTeA), and perfluoroundecanoic acid (PFUnA), exhibited biomagnification potential (TMF = 1.8 - 4.2, $p < 0.05$), whereas 5 pharmaceuticals (phenazone, progesterone, venlafaxine, levamisole, and lidocaine) and 1 personal care product metabolite (galaxolidone) showed biodilution potential (TMF = 0.4 - 0.6, $p < 0.05$).

3.01.T-02 Lanthanide Distribution in *Daphnia magna* Via Nano-Sxrf Imaging

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Rare earth elements (REE) are essential for many new technologies. Their increased application since the 1990s have led to elevated levels in aquatic system, and understanding their interaction with biological systems is an important area of research. Tests with daphnids (water fleas) let assume, that e.g. lanthanum toxicity could be similar to copper. Because of a number of chemical properties between Ln^{3+} and Ca^{3+} , Ln can block calcium channels and they interfere with signal recognition pathways and enzyme activity. All these toxic effects require uptake and potentially accumulation of REE into cells and tissues. But few data are available on the distribution of REE in organisms after exposure and on accumulated concentrations. In order to improve understanding of their bioavailability, the accumulation of two REE (La and Gd) in juvenile *Daphnia magna* has been measured at the NANOSCOPIUM hard X-ray scanning nanoprobe beamline of the synchrotron SOLEIL (Saint-Aubin, France). Neonates of less than 24h were exposed to 15 mg/L of La or Gd for 48 and 72h. At the end of the test, the survival organisms were dehydrated through an acetone-water series and dried in HDMS (1,1,1,3,3,3-hexamethyldisilazane) in order to be analysed by synchrotron nano-XRF (nano-SXRF). The measurement was performed with an incident monochromatic X-ray beam of 17.02 keV energy, producing nano-SXRF spectra for every pixel. The primary results showed a difference of distribution between La and Gd in the organism. Gd seemed to accumulate more than La and tended to be distributed in tissues. The accumulation increased over the exposure time and a stronger accumulation in the carapace was found at 72h compare to 48h. This may be due to the timing of the moulting. The first moult occurs in neonates before being exposed and the second after 48h. For 72h of exposure, La was found in the intestine tract and had a solid appearance in the hindgut. The ingestion of La was probably possible because of its precipitation either in the medium or in the animal's intestine itself. According to the speciation modelling by Geochemist Workbench, La tends to complex and precipitate more easily than Gd in solution conditions. Any solid matter generated might be ingested by the daphnids while the free ions may be taken up into the cells by e.g. Ca-channels. In conclusion, the accumulation of La and Gd in daphnid could depend on their speciation and free ion concentration.

3.01.T-03 Anionic and Cationic Organic Compounds Show Distinct Bioaccumulation in Rainbow Trout Cell Cultures

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The assessment of chemicals for their bioaccumulative potential requires *in vivo* testing with fish. These tests are resource intense, costly, time consuming and of ethical concern due to the sacrifice of animals. Therefore, alternative *in vitro* models are being sought to replace these tests. We have started to explore how well rainbow trout (*Oncorhynchus mykiss*) cell lines, in particular RTL-W1 (liver) and RTgill-W1 (gill), can predict the bioaccumulation and biotransformation potential of ionizable organic compounds (IOC) in fish. IOC comprise a large proportion of the chemicals in commerce and are ubiquitously detected in the environment and biota. Based on the availability of high quality *in vivo* data and the substances' charge state at physiological pH, four anionic and three cationic compounds were selected: Pentachlorophenol (PCP, CAS 87-86-5), Diclofenac (DCF, CAS 15307-79-6), Teclotalam (TT, CAS 76280-91-6) and Benzotriazol-t-butyl-hydroxyl-phenyl propanoic acid (BHPP, CAS 84268-36-0); and N,N,N-trimethyltetradecylamine (Q14, CAS 4574-04-3), N,N-dimethyldecylamine (T10, CAS 1120-24-7) and N-methyldodecylamine (S12, CAS 7311-30-0). First, non-toxic chemical concentrations for each compound were determined using the acute cell toxicity assay according to OECD TG249. Resulting exposure concentrations for bioaccumulation assessment ranged from 5 $\mu\text{g/L}$ to 200 $\mu\text{g/L}$, i.e. never higher than 1 μM . The kinetic experiments were performed over 48h and 72h for anions and cations, respectively, with a total of 6 sampling time points. Cell, medium and plastic fraction were sampled separately at each time point and measured using high resolution tandem mass spectrometry after online solid phase extraction (for anions) or with direct injection of extracts (for cations). The anionic compounds, given as a fraction of the total compound mass in the test system, accumulated in the following order in RTL-W1 cells: BHPP (6%) > TT (4%) > DCF and PCP (below limit of quantification, 0.07 to 0.45% respectively). In contrast, the thus far tested cations were associated with cells to a much larger extent, measured in RTgill-W1 as follows: 43% for T10 and 91% for S12. A comparison of the compounds' baseline bioconcentration factors and the *in vitro* derived steady-state bioconcentration factors gave a positive correlation ($R^2 = 0.79$), with deviations being within the 10-fold range of the line of unity.

3.01.T-04 Assessing Bioaccumulation of Silver Nanoparticles in Freshwater Benthic Invertebrates: From Single-Species to Mesocosm Approaches

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A sustainable nanotechnology development requires a robust environmental risk assessment. In line with this, studies on the

toxicokinetics and bioaccumulation of engineered nanomaterials (ENMs) have been highly requested by regulatory bodies. Aiming to contribute to this, the present study focused on understanding the toxicokinetics of different pristine silver nanoparticles (Ag NPs), silver sulfide nanoparticles (Ag₂S NPs, used as model of an environmentally aged Ag NP form) and AgNO₃ in the ecological relevant freshwater benthic species *Physa acuta* (pond snail), *Chironomus riparius* (non-biting midge) and *Girardia tigrina* (planarian). An integrated methodology was used, from lower (single-species bioaccumulation tests using different exposure routes) to higher tier (mesocosms, simulating a freshwater stream environment) approaches. Single-species tests with *P. acuta* revealed fast uptake and elimination of Ag from Ag₂S NPs in all experiments, and water exposure was the predominant Ag uptake route. *C. riparius* larvae consistently revealed higher Ag uptake upon exposure to Ag₂S NPs. In the individual chironomid exposures to water and sediment, Ag uptake was better explained by exposure to water than from the ingestion of sediment particles, while upon food exposure the larvae only revealed Ag uptake in the Ag₂S NP treatment. The Ag transfer from larvae to adult midges appeared to be limited. Planarians accumulated Ag from the food in Ag₂S NP and AgNO₃ treatments, uptake being higher for AgNO₃ exposures. The three species bioaccumulated Ag in Ag₂S NP and AgNO₃ exposures in the mesocosm test but showed higher internal Ag concentrations upon exposure to AgNO₃. The uptake observed in the Ag₂S NP treatment was likely in the particulate form, showing the bioavailability of this more environmentally persistent and relevant Ag nanoparticulate form in a more realistic exposure scenario. Single-species tests underestimated bioaccumulation when compared with mesocosms, albeit in some cases similar uptake patterns were observed. No apparent risk for biomagnification was observed in the food chain *P. acuta* to *G. tigrina* upon exposure to Ag₂S NPs in single-species and mesocosm tests. This work provides important data for modelling the potential exposure and bioaccumulation of relevant Ag forms in freshwater benthic environments and may be useful for predictive models for nanoregulation purposes, contributing to improving the environmental risk assessment of ENMs.

3.01 Advances in Bioaccumulation Science and Assessment (Part II)

3.01.T-06 Using In-Vitro Biotransformation Rates to Estimate the Bioaccumulation Potential of 6 Different Siloxane Compounds

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Siloxanes are produced globally in high volumes and are considered substances of concern in the EU. Siloxanes pose a unique challenge to assess for their environmental and human health impacts due to their superhydrophobicity, high volatility, and their universal presence, including their use in analytical instrumentation. Using OECD Test Guideline 319B to conduct the in-vitro studies, we looked at the depletion rates of 6 different siloxanes in both Rainbow trout and mammalian sub-cellular fractions. Octamethyltrisiloxane (L3), decamethyltetrasiloxane (L4), dodecamethylpentasiloxane (L5), octamethylcyclotetrasiloxane (D4), Decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) biotransformation rates were determined in both aquatic and mammalian liver S9 fractions and incorporated into in-vitro to in-vivo extrapolation models (IVIVE) to predict the bioconcentration factors of the 6 siloxanes. Each siloxane was run separately in replicates of 3, and each reaction vessel contained both Phase I and Phase II biotransformation enzymes and co-factors to help catalyze the reactions. Pyrene was run as a positive control to ensure the enzymes were active and functional. A heat-inactivated negative control was run to ensure that any loss observed in the reaction vessel was due to biotransformation. In-vitro data are compared against measured dietary bioaccumulation data in trout with the hopes to further our understanding of the role hepatic biotransformation has on bioaccumulation and the uncertainties or error that come with running extrapolation models. Additionally, the study hopes to better understand the differences between various species and their ability to metabolize xenobiotic compounds.

3.01.T-07 An Empirical Regression Model Based on Trout Liver S9 In Vitro Intrinsic Clearance and Log K_{ow} to Predict Bioconcentration Factors in Fish

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Bioaccumulation in aquatic species is a critical endpoint in the regulatory assessment of chemicals which usually involves the determination of the bioconcentration factor (BCF) in fish. The *in vitro* assay to determine *in vitro* intrinsic clearance ($CL_{IN\ VITRO,INT}$) in liver S9 fractions (RT-S9) from rainbow trout (OECD TG 319B) has been validated to refine BCF predictions using *in vitro-in vivo* extrapolation (IVIVE) models. Although incorporation of measured *in vitro* clearance in IVIVE models does improve BCF predictions, a tendency for overprediction was shown for different chemicals. The goal of this study was to establish a regression model to predict BCFs based on empirical data. As training set, 40 chemicals, including 27 fragrance chemicals, with RT-S9 *in vitro* intrinsic clearance and *in vivo* BCFs determined in different fish species (OECD TG 305) were used. A simple regression model ($\log BCF = a \times \log K_{ow} + b \times \log CL_{IN\ VITRO,INT}$) was built by estimating the parameters *a* and *b* by statistical fit based on *in vivo* BCFs, measured $CL_{IN\ VITRO,INT}$ in RT-S9 and $\log K_{ow}$. The regression analysis was performed with multiple datasets including species-matched subsets to evaluate the robustness of the estimation of the parameters *a* and *b* and the prediction of BCFs. Both parameters have a high statistical weight confirming the strong positive contribution of $\log K_{ow}$ and the impact of the $CL_{IN\ VITRO,INT}$ to reduce the predicted BCF. A leave-one-out-analysis was performed on the training set. The predictions for the left-out chemicals using the respective 40 submodels are very similar to the predictions with the regression equation based on the complete training set indicating that the empirical regression model performs well. As an external test set for model validation, additional $CL_{IN\ VITRO,INT}$ were determined or taken from the literature for 20 chemicals with *in vivo* BCFs. Geometric mean mispredictions (i.e. predicted BCF / *in vivo* BCF) of predicted BCFs with the regression equation were in the same range or slightly lower compared to commonly applied IVIVE models. Species-matched regression models (n=18 for trout, n=21 for carp) did not result in significant improvements of BCF predictions. The empirical regression model could be used

complementary to current IVIVE models to predict BCFs based on *in vitro* intrinsic clearance for bioaccumulation assessment and can overcome ambiguities such as the choice of the correct k_1 estimation.

3.01.T-08 Investigating the Applicability of Laboratory Derived Toxicokinetics to the Field

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Knowledge on bioaccumulation and biotransformation of organic micropollutants are essential to link exposure to effect and to designate appropriate environmental quality standards. Bioaccumulation and biotransformation of organic micropollutants in aquatic organisms have been investigated in laboratory experiments extensively. For several micropollutants, especially pesticides, however, the measured internal concentrations in gammarids in field trials exceeded the predictions based on the laboratory data, sometimes by multiple orders of magnitude. In this work, we tested the applicability of laboratory derived toxicokinetic rate constants for caged gammarids deployed in a small Swiss stream known for high pesticide loads. Using an automated mobile LC-ESI-HRMS/MS system, the aqueous concentrations of 49 pesticides were measured at high temporal resolution throughout several rain events during the application season (every 20 min for 1 month). The water concentrations from this study were then employed to model the whole body concentration of gammarids using a one-compartment toxicokinetic model with toxicokinetic rate constants derived in an accompanying lab study. This data was then compared to the measured whole body concentration of the caged gammarids. Furthermore, the pesticide concentrations in the stream sediment, dietary source (leaves) as well as in suspended particles during rain events were measured and used to model their effect on the bioaccumulation of the pesticides. Our results show that the internal pesticide burden of gammarids is highly dynamic, with the aqueous exposure as the main influencing factor. While the laboratory derived toxicokinetic rate constants predicted the temporal trends in whole body concentrations reasonably well, the values were systematically underestimated over the whole time period. The highest underestimation occurred shortly after rain events with the model underestimating the pesticide concentrations by a factor of up to 31 ± 3.0 . Finally, we demonstrated that while uptake from sediment does not contribute significantly to the total body burden even under worst case uptake assumptions, the suspended solids had considerably higher pesticide concentrations than the sediment and could explain the observed underestimation after rain events partially. Further possible reasons such as decreased biotransformation under field conditions or concentration dependence of the toxicokinetics are currently explored.

3.01.T-09 Development and Testing of an In-Vitro S9 Rat Liver Bioassay for Assessing Biotransformation Rates of Neutral Hydrophobic Organic Chemicals in Air-Breathing Organisms of Terrestrial Food-Webs

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In vitro biotransformation assays combined with in vitro-in vivo extrapolation (IVIVE) is a relatively novel approach to bioaccumulation assessments that can reduce cost, effort, time and animal testing and accelerate the assessment process. The Organization for Economic Co-operation and Development (OECD) has developed test guidelines 319B to determine in vitro biotransformation rates in fish using liver S9 of rainbow trout. However, similar protocols are not available for mammalian bioaccumulation assessments and in vitro liver S9 data for neutral hydrophobic substances with a high bioaccumulation potential in air-breathing organisms are largely unavailable. The objective of this study is to develop and test a refined protocol for in-vitro testing of biotransformation rates in rats using rat liver S9 that might be useful for bioaccumulation screening of neutral hydrophobic organic chemicals in air-breathing organisms. The framework has 4 components, including (a) S9 preparation and characterization, (b) preliminary experiments to optimize test conditions, (c) final experiments with multiple independent tests to determine in vitro biotransformation rates in a standardized way, and (d) analysis of assay results for bioaccumulation screening. The refined protocol was tested and evaluated using 14 test chemicals with a variety of chemical structures and biotransformation capacities. In vitro biotransformation rate constants (k_r) were measured and extrapolated to whole organism biotransformation rate constants (k_{met}) using 4 previously reported IVIVE models. Lipid-normalized biomagnification factors (BMF_L) were then calculated using a rat bioaccumulation model. The calculated total elimination rates were compared to available in vivo data. The results indicate that (a) measured in vitro depletion rates were highly reproducible in independent tests; (b) extrapolated whole organism biotransformation rates were similar among various IVIVE models; (c) calculated total elimination rate constants were in good agreement with reported in vivo data; and (d) application of the developed methodology shows that a substance is not expected to biomagnify in rats (i.e., $BMF_L < 1$) when in vitro $k_r > 0.3 \text{ h}^{-1}$ in rat liver S9. However, poorly metabolizable substances with an in vitro $k_r < 0.3 \text{ h}^{-1}$ in rat liver S9 are not expected to biomagnify if they have a low $\log K_{OA}$ (< 5), or a very low or very high $\log K_{OW}$ (< 1 or > 9), or exhibit a low dietary uptake efficiency ($< 5\%$).

3.01 Advances in Bioaccumulation Science and Assessment (Virtual Only)

3.01.V-01 Efficiency of Varied Xenobiotic Detoxification Biomarkers in Highlighting the Exposure of *Daphnia magna* to an Organophosphate Pesticide

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Freshwaters are among the most stressed ecosystems worldwide. Their increased overexploitation generates various anthropogenic stressors for living organisms. Among others, one stressor is the pollution by chemicals. Aquatic macroinvertebrates play an important role in aquatic food webs and are highly sensitive to chemicals. They can therefore be used as sentinel organisms for highlighting the deleterious effects of these compounds. The study of the biomarkers induced in these

organisms is of great interest for assessing the effects of low and chronic concentrations of compounds. In this study, we aim in finding out the most sensitive detoxification biomarker in *Daphnia magna* as a potential candidate for laboratory and *in situ* ecotoxic assessment. Cytochrome P450 system mostly operates in first step xenobiotic detoxification in aquatic invertebrates. The ability to measure this mixed function oxidase activity allows in understanding responses to chemical stressors. However, existing measurement of cytochrome P450 activities research for aquatic macroinvertebrates is limited with patchy and variable success. Our study is investigating several P450 biomarkers occurring during a process of detoxification on *D. magna*. Present work improves knowledge about P450 enzymatic biomarkers, specificity and accuracy to *D. magna* exposed to organophosphate chemicals and finally empowers determination level of aquatic macroinvertebrates' exposition to chemicals. Indeed, comparing and testing methodologies in laboratory is a crucial previous work for highlighting effects in the field. The main objective of the present work was therefore to test diverse cytochrome P450 enzymatic biomarkers (EROD, MROD, ECOD, APND and ERND) and to establish a suitable methodology for aquatic macroinvertebrates. Furthermore, our study investigated P450 biomarkers signal detection function of exposure time with the aim of understanding xenobiotic detoxification pattern through time. First, we tested these 5 biomarkers in *Daphnia magna* exposed for 5 days at 9 different concentrations of an organophosphate pesticide (diazinon). EROD, ECOD and APND showed a higher sensitivity. Secondly, we tested selected biomarkers, EROD, ECOD and APND in *D. magna* exposed for 1 to 5 days. Results are currently being analyzed.

3.01.V-02 Evaluating the Long-Term Toxicity and Bioaccumulation of Gold Nanobiomaterials in Fish

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Nanobiomaterials (NBMs) are receiving much attention because of their application in biomedical devices. However, their production and use also imply the risk of delivery to the environment. The aim of the present study was to evaluate the potential effects of gold nanoparticles (Au NP) in *Oncorhynchus mykiss* after long-term exposure via water. Effects were evaluated at two levels, the growth effect in juveniles (OECD TG215) and the Au bioaccumulation in fish and fish tissues (OECD TG305). Au NP (5-20 nm) with citrate as capping agent and spherical shape was supplied by Colorobbia Consulting SRL (Italy) as a liquid stock suspension. Working solutions (growth: 0, 0.05, 0.15, 0.5, 1.5, and 5 mg/L; bioaccumulation: 0, 0.5 and 5 mg/L) were prepared by direct pipetting of the stock in aquarium water. Water was renewed every two days. The exposure period lasted for 28 days in both assays, followed by a depuration phase of 14 days (water without NBM). For the growth assay, 9 fish were weighted at the beginning of the experiment and introduced in each aquarium (6 in total). Fish were weighted and fed at a ration of 4% body weight/day. Animals were weighted again after 14 days and at the end of the exposure. Tissues were frozen at -80°C to study different markers of toxicity. For the bioaccumulation (minimized test) assay 3 aquaria were used with 16 fish each. Four fish were sampled at time 0, 14 and 28 days of exposure and time 7 and 14 of depuration. Carcass and tissues (liver, gills, intestine, stomach and muscle) were digested using aqua regia, Au was analysed by ICP-MS. The differences between the measured and the nominal concentrations of Au in water were lower than 20 %. There were no effects on the fish welfare, neither significant differences in growth among groups. The possible sublethal effects are still under study. Au levels could be measured in different tissues at the two exposure levels. There was a reduction in Au levels in the whole body after 7 days of depuration with a complete elimination after 14 days. Muscle was not a tissue target of Au, but gills, stomach and intestine showed to be the principal receptors and accumulated the biggest amount of metal, followed by liver. After 14 days of depuration, gills and liver still showed high levels of Au. In conclusion 14 days of depuration were not long enough to assure the complete elimination of Au from gills and liver at the two exposure levels. *Acknowledgement* – H2020 project BIORIMA 760928

3.01.V-03 The Effect of Sulfidation and Soil Type on the Uptake of Silver Nanoparticles in *Enchytraeus crypticus*

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AgNPs are used in a wide range of daily products leading to their emission to waste water treatment plants (WWTP). Due to the presence of a large proportion of sulfide and the reduced conditions during wastewater treatment, Ag₂S is expected to be formed from Ag ions or AgNPs. The application of sewage sludge on agricultural land triggers concerns about exposure of terrestrial organisms to pristine or sulfidized AgNPs. Therefore, hazard assessment must be accurately performed. This study determined the uptake and elimination kinetics in *Enchytraeus crypticus* of different Ag forms: paraffin-coated 3–8 nm, PVP-coated 50 nm, and 60 nm AgNPs, 20 nm Ag₂S NPs PVP-coated and AgNO₃. The Ag₂S NPs were synthesized to simulate aged AgNPs after passing through a WWTP, while the other NPs were tested as manufactured. To assess the effect of soil type on the bioavailability and uptake of the Ag₂S NPs as the most environmentally relevant Ag species, animals were exposed through three different soils, Dorset, Woburn, and Lufa 2.2 (the same was carried out for AgNO₃). In all tests, *E. crypticus* was exposed to soil spiked at 10 mg Ag kg⁻¹ dry soil for 14 days and then transferred to clean soil for a 14-day elimination phase. During both phases, animals were sampled from three replicate jars on days 1, 2, 4, 7, 9, 11, and 14, kept overnight to purge their gut and frozen (-20 °C), dried, digested and analysed for Ag by graphite furnace Atomic Absorption Spectrophotometry. A first-order one-compartment kinetics model was used to describe Ag accumulation in the animals. The uptake rate constants were similar for 3–8 nm and 60 nm AgNPs and AgNO₃, but significantly different between 3–8 nm and 50 nm AgNPs, so, Ag bioavailability was influenced by its form and characteristics. Uptake and elimination rate constants of Ag significantly differed between the test soils. For Ag₂S NPs the Ag uptake and elimination rate constants were significantly lower for Dorset than for Woburn and with no significant differences between the estimated kinetics for Lufa 2.2 compared to the other two soils. For AgNO₃, significantly higher uptake and elimination rate constants in enchytraeids were found in the sandy and acid Dorset soil compared to the other soils. Therefore, soil

properties affect the bioavailability of Ag directly and bioaccumulation indirectly by affecting the transformation and distribution of Ag in soil and pore water. The kinetics of Ag₂S NPs cannot be predicted by those of AgNO₃.

3.01 Advances in Bioaccumulation Science and Assessment (Poster)

3.01.P-Mo094 Sorption of Three Pesticides to the Seaweeds *Ulva Lactuca* and *Sargassum muticum*

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Seaweed receives more and more global attention as a driver of a blue economy. It has the potential to deliver more food to an increasing world population and can provide basic ingredients for medicine, cosmetics, and even fuel. As a result, seaweed is being cultured in estuaries, open seas, or ponds. Seaweed grows best under nutrient-rich productive circumstances but here exposure to contaminants, such as pesticides, is likely. Depending on their hydrophobicity, an association of pesticides with seaweeds can occur. Although potentially problematic for some commercial uses, this also presents the possibility to use seaweed for remediation of effluent water from the pond- or tank-based aquaculture systems. To be able to predict associated (environmental) risks, we recommend extending existing models (e.g. the ERA-AQUA model) with a seaweed compartment. In order to assess seaweed-pesticide interactions, so-called sorption coefficients are required. Unfortunately, there is a general lack of sorption, uptake, bioaccumulation, and effects studies under laboratory conditions for seaweed species. In order to fill part of this data gap, a study on the sorption of three pesticides to the seaweeds *U. lactuca* and *S. muticum* was performed using a batch method according to OECD-106 at one concentration level. Three pesticides were selected to cover a representative range of expected sorption behavior, using sorption to the organic matter in soil K_{om} as a determinant. The K_{om} values of Thiamethoxam, Diazinon, and Chlorpyrifos are 33, 353, and 2072 L/kg, respectively. Results of the sorption experiments will be presented. In future work, the pesticide sorption coefficients determined for seaweeds can be used in risk assessment by extending existing models like ERA-AQUA with a seaweed compartment to calculate pesticide content of seaweed and pesticide concentrations in the pond or tank water and in effluent released from aquaculture systems.

3.01.P-Mo095 Trophic Magnification of Perfluoroalkyl Substances Within a Terrestrial Avian Food Web

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The primary endpoints and criteria currently used in North America to define a substance as bioaccumulative include log octanol-water partition coefficients (log KOW) >5 and/or bioconcentration or bioaccumulation factors (BCF or BAF) >5,000. However, these endpoints limit our ability to identify substances that may be bioaccumulative in air-breathing organisms or terrestrial ecosystems since they do not account for chemical exposure from air or in air-breathing organisms. In addition, these endpoints, particularly KOW, are not suitable for understanding the bioaccumulation potential of perfluoroalkyl substances (PFAS) within terrestrial systems because KOW cannot be reliably measured for ionic surfactants. To establish effective guidelines that protect terrestrial species, there is a need to further investigate the bioaccumulation process of PFAS in terrestrial organisms. Thus, we assessed biomagnification of a suite of PFAS [all perfluoroalkyl acids (PFAAs)] in an urban terrestrial food-web that included an avian apex predator, the Cooper's hawk (*Accipiter cooperii*). We analysed samples of Cooper's hawk eggs, songbirds, invertebrates, soil, and air samples for concentrations of PFAAs. Each organism sample was analysed for protein and phospholipid content to account for differences in sorbing matrices. Using stable isotope analysis of ¹³C and ¹⁵N signatures, we estimated the trophic position of each organism. Trophic Magnification Factors were determined using three approaches: 1) wet weight concentrations (TMFW); 2) concentrations normalized based on tissue composition in each organism (i.e., protein, lipid, and phospholipid; TMFN); and 3) concentrations expressed as thermodynamic activities (TMFA). TMFWs of PFAAs ranged from 0.75 to 6.8 and TMFNs ranged from 0.81 to 7.5, indicating that most PFAAs are biomagnifying in this urban terrestrial system.

3.01.P-Mo096 A Generic Model Based on the Properties of Nanoparticles and Cells for Predicting Cellular Uptake

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Nanoparticles (NPs) are widely used in industry and technology due to their small size and versatility, which makes them easy to enter organisms and pose threats to human and ecological health. Given the particularity and complex structure of NPs, statistical models alone cannot reliably predict uptake. Hence, we developed a generic model for predicting the cellular uptake of NPs with organic coatings, based on physicochemical interactions underlying uptake. The model utilized the concentration, experimental conditions and properties of NPs viz. size, surface coating and coverage. These parameters were converted to surface energy components and surface potentials, and combined with the components and potential for a cell membrane. For NPs uptake, we constructed energetic profiles and barriers for adsorption and permeation onto/through cell membranes. The relationships derived were compared to experimental uptake data. The model provided accurate and robust uptake estimates for neutrally charged NPs and six different cell types. Our model also has some applicability for charged NPs, which may indicate significance that physicochemical interactions is common in different cellular uptake pathways of various NPs. We envision that the model provides a reference for cellular accumulation of NPs and (ecological/human) risk assessment of NPs or microparticles.

3.01.P-Mo097 Influence of PFOs on the Bioaccumulation of Emerging Contaminants in Crops Cultivated Under Soil and Soilless Conditions

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The Mediterranean is a region of substantial agriculture production that faces concurrent environmental stresses and freshwater pollution given the occurrence of Pharmaceuticals and Personal Care Products (PPCPs). Among these pollutants, the surface-active substances have been suggested to enhance the bioavailability of other PPCPs. This research evaluates a comparative uptake and translocation assessment of irrigation exposure to atenolol (ATN), carbamazepine (CBZ) and triclosan (TCS) alone vs. combined with perfluorooctanesulfonic acid (PFOS) under semi-field (i.e. soil experimental set) vs. hydroponics (i.e., soilless experimental set) growing conditions with lettuce, radish and tomato plants. Both experimental sets revealed efficient root uptake and translocation for the three PPCPs regardless of their co-existence with PFOS. The overall results of the uptake and translocation of the PPCPs in the lettuce and tomato plants suggested a simultaneous treatment-plant organ interaction, which was not affected by PFOS being present in both experimental sets. These observations support the hypothesis of factors other than PFOS being responsible for the differential bioaccumulation and translocation potentials seen in both experimental sets. However, the radish plants co-irrigated with PFOS brought about increased movement of PPCPs from roots to aerial parts. These results support the notion that factors inherent to the physiological characteristics of this root vegetable contributed to PPCPs' increased tendency to move from roots to aerial parts. This work has been supported by Spanish Government Grants (CTM-2013-44986-R and CTM-2014-52388-R) funded by MCIN/AEI/ 10.13039/501100011033 and the ERDF.

3.01.P-Mo098 How Can Passive Sampling Be Used to Help Understand POP Bioaccumulation in Fish?

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Hexachlorobenzene is ubiquitously distributed in the environment and is assumed to mildly biomagnify in aquatic foodwebs. The use of trophic magnification factors (TMF) in the procedure to compare contaminant levels in biota with Water Framework Directive (WFD) Environmental Quality Standards has been proposed. An understanding of biomagnification and reliable selection of TMF is therefore crucial in this framework. Passive sampling for nonpolar non-ionised chemicals has been the subject of much progress over the last two decades. Latest advances include the ability through the use of lipid-polymer partition coefficients to estimate equivalent contaminant concentrations in lipids at equilibrium with the water (sampled by the passive sampler). This can in turn help with understanding bioaccumulation processes through comparisons with actual biota data. Here we compare datasets of penta- and hexachlorobenzene (PeCB and HCB) concentration ratios (i) from passive sampling in water and (ii) in freshwater and marine fish acquired through routine monitoring programmes in Norway in the last 5-10 years. In our procedure, PeCB is used for benchmarking bioconcentration in fish and observed HCB/PeCB ratios in fish are compared with ratios expected in the case of (a) HCB bioconcentration only or (b) biomagnification using published TMFs. Our results show that we cannot conclude that HCB biomagnifies in fish species that would be used for WFD monitoring in Norway. Finally, fish-water chemical activity ratios for HCB and PeCB as well as for polychlorinated biphenyls (PCBs) calculated for coastal sampling locations where biota and passive sampling were conducted alongside rarely exceed unity for cod. Implications are discussed.

3.01.P-Mo099 Contaminants of Emerging Concern in Glass-Eel (*Anguilla anguilla*): Occurrence, Bioconcentration and Biotransformation

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Undoubtedly, the presence of contaminants of emerging concern in the aquatic environment has a direct impact on water-living organisms and can alter their living functions. These compounds are often metabolized and excreted but they can also be accumulated and spread through the food chain. The metabolized contaminants can also lead to the formation of new compounds with equal or higher toxicity and bioaccumulation potential as the parent compound. In this work we have studied the occurrence, bioconcentration and biotransformation of CECs in glass-eels (*Anguilla anguilla*) using UHPLC-Q-Exactive Orbitrap. We first carried out an environmental risk assessment of the WWTP effluent that releases directly into the estuary of Adour (Bayonne, Basque Country, France). The risk quotients of every detected contaminant were calculated and three ecotoxicologically relevant contaminants were chosen to perform the exposure experiment: propranolol, diazepam and irbesartan. An experiment of 14 days consisting of 7 days of exposure and 7 days of depuration was carried out to measure the bioconcentration of the chosen compounds. The quantitative results of the concentrations in glass eel showed that diazepam and irbesartan reach BCF ? 10 at day 7, but both compounds are completely eliminated after 7 days of depuration. On the other hand, propranolol's concentration remains constant all along the experiment, and its presence can be detected even in the non-exposed control group. Two additional suspect screening strategies were used to identify both the metabolization products of the target compounds, and other xenobiotics already present in wild glass eels. Several metabolites were annotated for propranolol and diazepam, whereas none was detected for irbesartan. The xenobiotic screening confirmed the presence of more xenobiotics in wild glass eels, prominent among them, the pharmaceuticals exemestane, primidone, iloprost and norethandrolone, the industrial products caprolactam and diethylenetriamine or THC among the recreational drugs.

3.01.P-Mo100 Uptake of Pharmaceuticals and Personal Care Products by Crops Irrigated With Reclaimed WATER in a Mediterranean Scenario

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Water scarcity is a natural condition in the Mediterranean rim countries. In this region, reuse of reclaimed water (RW) from wastewater treatment plants (WWTPs) is becoming a potential source for highly water-demanding activities such as agriculture. Considering a Mediterranean scenario, we investigated the plant uptake and translocation of three representative pharmaceuticals and personal care products (PPCPs) (atenolol -ATN-, carbamazepine -CBZ- and triclosan -TCS-) typically present in RW samples from a WWTP located in an urban area in Spain. We then assessed the potential risk to humans from plant consumption. To consider two worst-case scenarios the contaminants were spiked at two levels in the RW samples. Three plant models (lettuce, maize and radish) were grown outdoors and irrigated with four treatments: tap water; RW samples, and the two spiked RW samples. Results revealed an efficient root uptake for the three PPCPs regardless of plant species and fortification level. Different bioaccumulation and translocation potentials of the three PPCPs were seen in the aerial plants organs. We hypothesized that several factors, including physical-chemical properties of the PPCPs and physiological plant variables, could be responsible for the differential accumulation and translocation potentials observed. These variables could be critical for crops irrigated with RW in regions with extended dry seasons, high solar incidence and low annual rainfall including those in the Mediterranean rim. The results suggested a negligible risk to humans from consumption of edible plants irrigated with RW samples with presence of PPCPs. This work has been supported by Spanish Government Grants (CTM-2013-44986-R and CTM-2014-52388-R) funded by MCIN/AEI/ 10.13039/501100011033 and the ERDF.

3.01.P-Mo101 Investigations on the Uptake and Accumulation of Wastewater-Borne Nanomaterials and Released Metals by the Benthic Amphipod *Hyalella azteca* Under Environmentally Relevant Conditions

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With an increasing range of applications, the diversity, production levels and use of engineered nanomaterials (ENMs) is growing at a global level. When ENMs are incorporated into consumer products, such as cosmetics, textiles and nanomedicine, a proportion can be released into wastewaters during use or end of life. After entering the wastewater stream, ENMs become primarily attached to the sludge flocs and are thus removed relatively efficiently by wastewater treatment plants (WWTPs). Nevertheless, small amounts of ENMs entering WWTPs can reach the environment directly via the effluent, attached to sludge flocs or even as free particles. Furthermore, the biosolids from WWTPs are often used as agricultural fertilizer and from there ENMs may enter aquatic systems via surface runoff. Relatively few data are available regarding the fate and transformation of ENMs released from WWTP and their potential bioavailability to organisms in different aquatic systems. Most studies on bioavailability or accumulation of ENMs have been performed using pristine ENMs, where important physicochemical transformation processes are rarely taken into account. A few studies have been conducted with artificial wastewater to simulate the transformation processes in WWTP, but mostly using high ENM concentrations. In this study, we used a pilot WWTP operating with real wastewater and spiked with low concentrations of Au, Ag or ZnO ENMs, to achieve sludge concentrations comparable to levels observed in the field. Concentrations of 1 (Au), 10 (Ag) and 100 (Zn) mg/kg dry mass of the digested sludge were produced. To allow the detection of the spiked ENMs at low concentrations in complex matrices, as well as to differentiate against the background, we used isotopically enriched ENMs (Ag and ZnO -NP). Methods for ENM extraction were developed and measurement of total metal and particle concentrations was carried out by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) and single particle-ICP-MS, respectively. The benthic amphipod *H. azteca* was used as model organism, as the uptake and detoxification mechanisms for metals in this species are well known and described. After exposures with effluent or biosolids from the pilot WWTP, we examined the uptake, accumulation and bioavailability of the wastewater borne ENMs and the respective metals. The obtained results were compared with further data from exposures conducted with pristine ENMs, the dissolved metals and different exposure conditions.

3.01.P-Mo102 Acute Aquatic Toxicity in Zebrafish and Bioaccumulation in Mussels of Antimony Tin Oxide Nanoparticles

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Antimony Tin Oxide (Sb₂O₅/SnO₂) is effective in the absorption of infrared radiation for applications such as skylights. This nanoparticle (NP) can be incorporated into film or sheet applications and provides infrared radiation attenuation while allowing for a transparent final product. Despite of being integrated in many products, including plastics, very scarce information is available regarding their ecotoxicity and bioaccumulation. In this work, after extensive characterization of the nanomaterials (DLS, Z-potential, TEM, SEM, XRD), we studied the acute toxicity exerted by commercial Sb₂O₅/SnO₂ in adults and embryos of

zebrafish (*Danio rerio*). Our results suggests that these NPs do not induce an acute toxicity on zebrafish, nor the adults, either the embryos. However, some sub-lethal parameters were altered; eye volume, heart rate and spontaneous movements. Finally, we studied the possible bioaccumulation of these NPs in aquacultured marine mussel *Mytilus* sp. as a potential bioconcentrator prior human intake. We performed a quantitative analysis by sp-ICP-MS and biodistribution analysis by TEM of the mussel's tissues. The results indicated that despite being scarce ($2.31E+06 \pm 9.05E+05$ particles/g), there is some accumulation of the Sb_2O_5/SnO_2 NPs in the mussel. No NPs could be observed in the tissues due to the relatively low concentration and no significant alterations were observed in the ultrastructure of the tissue or cells. In conclusion, commercial Sb_2O_5/SnO_2 NPs seem to be quite innocuous to aquatic organisms, however, the fact that some of the developmental parameters in zebrafish embryos are altered should be considered for further investigation. More in deep analysis of these NPs transformations in the digestive tract of humans, their bioaccessibility and bioavailability are needed to understand if their accumulation in mussels pose an effective risk to humans.

3.01.P-Mo103 Differences in Chemical Contaminants Bioaccumulation in Presence or Not of Microplastics in Mussels (*Mytilus galloprovincialis*)

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Persistent Organic Pollutants (POPs) are one of the most important indicators of the anthropic influence on the environment, specially on the biota as they are bioaccumulable and have recently shown to interact with microplastics (MPs) [1]. Mussels are suitable for in-lab aquarium experiments, such as bioaccumulation studies, which provide insight about the occurrence and fate of pollutants in the organisms. In the present study, bioaccumulation in *Mytilus galloprovincialis* of 20 POPs including pharmaceuticals and personal care products (PPCPs), pesticides, and perfluoroalkyl substances (PFASs) was assessed with or without the influence of MPs. Mussels (*M. galloprovincialis*) were distributed randomly in three groups: (B): control; (C): exposed to POPs; and (C+M): exposed to POPs+MPs (polyethylene). The experiment was carried out in two stages: exposure (0-28 days) and depuration (29-58 days). Visceral mass and haemolymph of the mussels were extracted separately, using QuEChERS and solid phase extraction. Extracts were analysed via UHPLC-MS/MS. Results showed that 3 PPCPs, 4 pesticides and 3 PFASs accumulated in visceral mass with bio-concentration factors (BCFs) ranging 6.7–15000 L/kg. In haemolymph, 2 PPCPs, 2 pesticides and PFPeA were detected with BCFs ranging 0.9–3.3 L/kg. Comparing C and C+M, MPs worked as a vector for the accumulation of the PFASs with higher BCFs in the presence of MPs. Furthermore, the elimination of PFDA and PFOS was slower in the mussels exposed to MPs. On the other hand, the pesticides terbuthylazine and chlorpyrifos showed lower BCFs and more rapid elimination in the mussels exposed to MPs. **Acknowledgements** This work has been supported by Grant RTI2018-097158-B-C31 funded by MCIN/AEI/ 10.13039/501100011033 and “ERDF A way of making Europe”. [1] Bhagat, J., Nishimura, N., Shimada, Y., 2021. J. Hazard. Mater. 405, 123913.

3.01.P-Mo104 Size- and Shape-Dependent Bioaccumulation Dynamics of Microplastic and Microrubber Particles in the Eastern Oyster, *Crassostrea virginica*

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Microplastics (MP) are ubiquitous in coastal waters and have been documented in filter-feeding organisms such as oysters. Along the South Carolina coast (U.S.) where the ecologically and economically important eastern oyster (*Crassostrea virginica*) forms large intertidal reefs, recent surface water surveys found that fibers, fragments, and microscopic tire particles represented 43.6%, 30.9%, and 17.7% of the total MP, respectively. The aim of the current study was to characterize the bioaccumulation dynamics of these three particle types in the eastern oyster. In the laboratory, oysters were exposed to either purple polyethylene fibers, green nylon fragments, or micronized crumb rubber at a concentration of 5000 MP/L. Oysters (n=8 to 10) were sacrificed after 0, 24, 48, and 96 hours of exposure to characterize MP uptake. Following 96 hours of exposure, remaining oysters were transferred to MP-free seawater and sacrificed at 24, 48, and 96 hours (n=8 to 10) to characterize MP depuration. Oysters accumulated significantly longer fibers, smaller fragments, and smaller crumb rubber particles relative to the average sizes to which they were initially exposed. For fibers and fragments, MP levels increased during the uptake phase in a hyperbolic fashion with apparent steady states being achieved at 96 h at 1.61 ± 0.56 particles/g w.w. (mean \pm SE) and 0.46 ± 0.08 particles/g w.w., respectively. For fibers, K_u was estimated to be 0.0057 ± 0.0016 mL/g*h (mean \pm SE), and K_d was estimated to be 0.0151 ± 0.0059 /h. For fragments, K_u was estimated to be 0.0016 ± 0.0003 mL/g*h (mean \pm SE), and K_d was estimated to be 0.0168 ± 0.0047 /h. For crumb rubber, levels increased during the uptake phase following a linear relationship with levels after 96-h at 3.62 ± 0.84 particles/g w.w. The K_u and K_d were estimated to be 0.0071 mL/g*h and 0.0067 ± 0.0033 /h, respectively. MP levels in oysters decreased during the 96-h depuration phase by 78.6% for fibers, 80.8% for fragments, and 64.2% for crumb rubber. The present study demonstrates accumulation of MP and microrubber in eastern oysters is size- and shape-dependent and depuration is an effective method to reduce MP levels. Although it has been asserted that bivalves make poor general bioindicators of MP pollution because of their particle selectivity, we suggest that the eastern oyster has the potential to be specific bioindicators for smaller, low aspect-ratio fragments and tire particles, and larger, higher aspect-ratio fibers.

3.01.P-Mo105 Environmental Occurrence of Organophosphate Esters and Micro-Plastics in Sediments and Benthic Organisms From the Loire Estuary (France)

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Organophosphate ester (OPE) flame retardants and plasticizers are organic plastic additives commonly incorporated in many industrial products in order to minimise fire risks and/or to improve some properties of manufactured products [1]. These

chemicals present multiple sources in the environment and are widely distributed in seas and oceans of the planet [2]. Another emerging threat to the marine environment is micro-plastics (MPs) due to substantial loading from rivers, coastal cities, and through maritime traffic and fishing activities [3,4]. Marine sediments are thought to be a sink for both OPEs and MPs. In addition, leaching from MPs could constitute in-situ sources of OPEs [2]. Therefore, determining their environmental occurrence in sediments becomes crucial to better understand their current stocks, the exposure of benthic organisms, and possible impacts on the global functioning of marine ecosystems. The Loire is the longest river in France representing an important freshwater discharge to the North-East Atlantic Ocean. Its estuary is considered as an ecosystem of high ecological value as well as an ocean/land interface for sea trade and associated industrial activities. Three sampling campaigns conducted in 2021-2022 allowed the collection of surface sediments and the deposit feeder clam *Scrobicularia plana* (*SP*) in four study areas subjected to different anthropogenic pressures. A large set of OPEs were quantified by isotopic dilution LC-MS/MS after thorough clean-up and MP abundance was determined after matrix digestion and polymer isolation and the fragments were characterized by μ -FTIR. Our results confirm the overall presence of most abundant chlorinated and non-chlorinated OPEs in sediments from the study area. Six out of the 11 monitored OPEs in biota were also detected in *SP*, at concentrations as high as 7 ng/g d.w for tris(chloro-2-propyl) phosphate (TCIPP). The OPE concentrations in sediment and geographical distributions are discussed with regards to MP abundances. A first estimation of the field-derived biota-sediment accumulation factors (BSAFs) of OPEs is also presented. These results emphasize the need to further explore the sources and fate of this class of compounds ubiquitous in the marine environment. **References:** [1] van der Veen, I., de Boer, J. Chemospher 2012, 88, 1119–1153; [2] Xie et al., Nat. Rev. Earth Environ, in press; [3] Lebreton et al. Nat. Commun. 2017, 8, ncomms15611; [3] Schmidt, et al. Environ. Sci. Technol. 2017, 51, 12,246–12,253.

3.01.P-Mo106 New Insights on In Vitro Biotransformation of Anticoagulant Rodenticides in Fish

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The assessment of the bioaccumulation potential of chemicals is an essential and mandatory part of their regulatory environmental risk and hazard assessment. Within the EU active substance approval under the Biocidal Products Regulation, the bioaccumulation potential of most anticoagulant rodenticides (ARs) was assessed from quantitative structure activity relationship (QSAR) derived bioconcentration factors (BCFs). *In silico* approaches are based on the experience that the BCF value of a substance is usually related to its lipophilicity and susceptibility to biotransformation. However, BCF estimations relying on the *n*-octanol/water partition coefficient neglect other driving forces concerning ARs. The majority of ARs is presumed to be ionizable under environmentally relevant conditions. Additional complicating factors are their stereochemistry and tissue specific protein binding to the target enzyme vitamin K epoxide reductase with the liver being the main organ of accumulation and storage. Moreover, QSARs often do not account for the contribution of fish metabolism on intrinsic clearance. So far, *in vitro* data on fish metabolism is rarely available for biocidal active substances such as ARs. In this study we present *in vitro* biotransformation rates of eight biocidal and one pharmaceutical anticoagulants in rainbow trout (*Oncorhynchus mykiss*) liver subcellular S9 fraction (RT-S9) determined following the OECD test guideline 319B method at two different incubation temperatures (i.e., 12 ± 1 °C and 23 ± 2 °C). Furthermore, we address challenges associated with the usability and interpretation of *in vitro* data to support the decision making within the regulatory bioaccumulation assessment in bridging the gap between *in silico* methods and *in vivo* studies. Overall, four of the tested substances (i.e., chlorophacinone, coumatetralyl, bromadiolone, and difenacoum) exhibited significant intrinsic clearance ($p < .001$) in the RT-S9 assay. The observed metabolism was (very) slow and clearance rates were temperature-dependent. For substances with otherwise scarce experimental data availability, the known presence or absence of *in vitro* intrinsic clearance in fish using the RT-S9 assay is expected to improve the decision base for regulators to request or avoid further *in vivo*/animal testing during environmental risk and hazard assessments.

3.01.P-Mo107 Tiered Approach to Assess Bioaccumulation in Air-Breathing Organisms

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Bioaccumulation assessment has focused mainly on aquatic species in the past, with the fish bioconcentration factor (BCF) as a widely accepted endpoint. The BCF is used as criterion in PBT and POP assessment. Although for many substances this is sufficient, some substances like endosulfan, many perfluorinated alkyl substances or highly lipophilic compounds, accumulate substantially in air-breathing organisms and may not be recognised as bioaccumulative if based on aquatic testing only. An ECHA working group including members from academia, industry and government (from Europe and Canada) are developing an approach to use toxicokinetic data for the prioritisation and assessment of substances, potentially bioaccumulating in air-breathing organisms. The working group proposes a tiered approach, where the first tiers can be used for (de)prioritisation and higher tiers include testing strategies that allow a definitive conclusion. The relevant assessment endpoint is the biomagnification factor (BMF), with a value greater than 1 indicating concern regarding bioaccumulation potential in the food chain. Other endpoints, such as elimination rates and biotransformation rates can be related to this BMF reference value. Tier 1 assessment compares a

chemical's properties (volatility, hydrophilicity, and susceptibility to biotransformation) with threshold values to separate chemicals that are potentially bioaccumulative from those that clearly will not be able to bioaccumulate. The subsequent tiers use data of increasing complexity, ranging from biotransformation rates estimated with quantitative structure activity relationships (QSARs), to in vitro test results combined with in vitro in vivo extrapolation (IVIVE), to biotransformation rates derived from in vivo studies for air-breathing organisms. A standard protocol for in vitro tests, similar to that currently available for fish in OECD TG 319 A and B, could also be developed for e.g. rat. The assessment in the highest tier involves in vivo testing with air-breathing vertebrates and therefore should only be applied as last resort. Existing guidelines which are being explored regarding their utility for bioaccumulation assessment (either as such or with modifications) include OECD TG 417 (toxicokinetic) and OECD TG 422 (combined repeated dose toxicity study). The use of food chain data can be very useful information to be considered in this tier while guidance on the use is needed.

3.01.P-Mo108 Bioaccumulation Assessment Methods for Hydrocarbons and Related Substances in Terrestrial Organisms

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Currently, there is an increased interest in the development of regulatory metrics for assessing the bioaccumulation of hydrocarbons and related substances in terrestrial organisms. This is due to a lack of regulatory methods and criteria for evaluating the bioaccumulation of substances in air-breathing organisms of terrestrial systems; the recognition that “low Kow-high Koa” substances exert a bioaccumulation behaviour in terrestrial organisms that cannot be adequately determined from bioaccumulation tests in fish; the finding that certain polyfluorinated organic chemicals bioaccumulate to a high degree without being lipid soluble; as well as the phase-out of animal studies for testing and the urgent need for an enhanced capacity for bioaccumulation testing at lower costs and within a shorter time frame. Fortunately, over the last few years, several new developments in bioaccumulation science have emerged that can contribute to the development of effective regulatory tools for the management of hydrocarbons and related substances in terrestrial wildlife. They include in-vitro biotransformation testing; the developments of in-vivo protocols for dietary bioaccumulation tests; the development of quantitative structure activity relationships (QSARs) for biotransformation and bioaccumulation; and the growing capacity to measure trophic magnification of substances in real-world food-webs. have provided a number of options for better assessment and management of hydrocarbons and other chemicals. It is the objective of this study to review these developments and evaluate their potential to generate bioaccumulation metrics and data for air-breathing organisms that have a strong scientific foundation and are informative and useful for regulations. The study explores in-vitro depletion tests and in-vitro-to-in-vivo extrapolation techniques to assess biomagnification factors in air-breathing organisms of terrestrial food-webs; in-vivo biomagnification factors for lipid soluble and non-lipid soluble substances; QSARs for biotransformation and biomagnification; and field derived trophic magnification factors (TMF). Merits and limitations of the various approaches to terrestrial bioaccumulation assessment are discussed and the potential for a weight-of-evidence approach drawing on available information from several sources is highlighted.

3.01.P-Mo109 High-Resolution Mass Spectrometry for the Metabolomic Profiling of Endometriosis Patients and Pregnant Subjects

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Metabolomic tools represent a step forward in clinical studies that help to clarify the underlying processes triggered by specific health-related conditions. These studies are mainly based on the comparison of the different metabolic fingerprints from biological samples obtained through advanced analytical techniques. In this line, liquid chromatography (LC) coupled to high resolution mass spectrometry (HRMS) have emerged as a promising tool in metabolomic studies due to its ability to identify untargeted compounds contained in complex samples with a high degree of precision. The most common biological matrix for metabolomics studies is urine, due to its easy and non-invasive collection. Thus, the goal of the present study was to assess the suitability of a simple salt-assisted liquid-liquid microextraction (SALLE) followed by a HRMS strategy for determining the metabolic profile of urine samples from endometriosis patients, pregnant and control subjects ($n = 10$). The SALLE procedure was applied in parallel with acetonitrile and ethyl acetate aiming to extract compounds with different polar character. HRMS data were compared with records from the spectral library METLIN and a threshold identity score of 80 was selected for further identification. Results showed the tentative presence of up to 167 compounds belonging to different classes. Differential compounds across conditions were mainly molecules involved within the endocrine system, such as pregnanolone sulfate or dibutyl phthalate, an endocrine disruptor, exclusively found in samples from endometriosis patients, whereas 11-deoxy-11-methylene-prostaglandin D2 or prostaglandin D2 were only present in samples from pregnant subjects. Results showed that the use of a simple sample preparation procedure followed by HRMS analysis evidenced the hormonal imbalance among the assessed conditions and point to specific molecules as responsible for these differences. Therefore, these results provide more evidence about the key role of HRMS in clinical biomonitoring studies and support its applicability for further studies. **Acknowledgments:** This research was supported by the Spanish Ministry of Science and Innovation PID2020-115871RB-I00

3.01.P-Mo110 A New Integrated Screening-Level Bioaccumulation Modelling Framework for Organic Chemicals

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Bioaccumulation (B) assessments can use various methods, data, and metrics. Measured B data for aquatic organisms are only

available for about 5% of the organic chemicals that require B assessment necessitating the use for models and new approach methods (NAMs) to address data gaps. There is now increasing recognition that B assessments should consider air-breathing organisms. Mechanistic mass-balance models have been developed for simulating the bioaccumulation of organic chemicals in aquatic and terrestrial organisms. While the partitioning of legacy neutral organic pollutants is often well characterized using octanol as a surrogate for lipids and other organic phases, this simplifying assumption is not appropriate for certain types of chemicals such as ionizable organic chemicals (IOCs). In particular, the charged form of IOCs shows relatively high affinity for phospholipids and in some case serum albumin. Approximately 45% of the organic chemicals that require regulatory evaluations are IOCs, therefore there is a need for evaluated B models for IOCs. Mass-balance models require biological and system properties as well as chemical-specific input parameters. Data gaps for physical-chemical properties can be addressed using quantitative structure-activity relationships (QSARs) and polyparameter linear free energy relationships (PP-LFERs). Data gaps for biotransformation rates can be addressed using in vitro methods and QSARs for estimating biotransformation rates. In this work we describe and evaluate a new screening-level bioaccumulation modelling framework for neutral and ionizable organic chemicals for a diverse range of representative aquatic and terrestrial organisms. The “B-Screen” model is developed in a tiered approach for input parameters allowing the user to use inputs at different level of complexity. The new model is integrated in the on-line Exposure And Safety Estimation (EAS-E) Suite platform (www.eas-e-suite.com) which can autoparameterize the model for >50K chemicals and facilitates its operation in the regulatory community. The B Screen model is consistent with the models built into the Bioaccumulation Assessment Tool (BAT Ver.2.0), such that higher tiered evaluations requiring multiple lines of evidence in a weight of evidence approach can evolve, if necessary, from the screening calculations provided by the B-Screening model. Details of the models, input parameters and case applications are presented.

3.01.P-Mo111 QSAR Models for the Prediction of Bioaccumulation in Aquatic and Terrestrial Organisms

Ester Papa, Linda Bertato and Nicola Chirico, University of Insubria, Italy

Experimental data related to metrics which describe the potential bioaccumulative behaviour of chemicals in different organisms and environments is important to support risk assessment procedures. Bioaccumulation (B) is a process by which the concentration of a chemical in an organism exceeds the concentration in its natural environment. B can be described by different metrics such as Kow, Koa, Bioaccumulation Factor (BAF), Bioconcentration Factor (BCF), Biomagnification Factor (BMF), Biota-Sediment Accumulation Factor (BSAF), Trophic Magnification Factor (TMF). Since experimental determination of these quantities can be time consuming and expensive, in terms of money, resources and animal lives, the maximization of the existing information and the use of alternatives strategies, such as QSAR models, are useful to fill data gaps and to estimate the properties of interest from the molecular structure of new and existing chemicals. In this work, some of the B metrics reported above, have been modelled by QSAR starting from data collected and curated from the literature or from online databases. The molecular structures represented by SMILES were standardized and used to calculate molecular descriptors, which are the independent variables necessary to develop multiple linear regression (MLR) QSAR models. Two heterogeneous sets of about 1400 and 300 chemicals respectively, were collected and curated in order to develop QSARs for the prediction of BCF and BMF in fish. These QSARs are characterized by good statistical performances (R^2 and Q^2 between 0.60 and 0.85). Additional QSARs were created for the BSAF measured in three organisms, belonging to either soil (*Lumbriculus variegatus*) or to sediment (*Monoporeia affinis* and *Lytechinus pictus*). R^2 and Q^2 values of these BSAF QSARs are in the range 0.70-0.91 and 0.62-0.87, respectively. The development of models for B in plants is ongoing and preliminary results are promising. All the models were developed considering the OECD requirements for model's transparency, statistical validity, and defined domains of applicability. The here proposed QSARs can be used to support the evaluation of the risk that chemicals may pose to different organisms in the environment, using multiple bioaccumulation metrics.

3.01.P-Mo112 The Bioaccumulation Assessment Tool (BAT, Ver.2.0): A Weight-Of-Evidence Framework for Bioaccumulation Assessment

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Bioaccumulation (B) assessments using various methods, data, metrics, and classification criteria are routinely conducted as a part of chemical regulatory programs. For some chemicals B assessments can be challenging because of the myriad of metrics and threshold values combined with the uncertainty and variability in measured data. The Bioaccumulation Assessment Tool (BAT) guides a user through the process of collecting and generating various lines of evidence (LOE) required for assessing the bioaccumulation of neutral and ionizable organic chemicals in aquatic organisms and homeotherms (mammals). The BAT Ver.2.0 provides a formal Weight of Evidence (WOE) framework for B assessment considering both aquatic and terrestrial data sources (lab, field, in silico) consistent with OECD WOE principles. The BAT allows the user to enter various B metrics (i.e., LOE) for aquatic organisms (fish and invertebrates) such as in vivo laboratory BCFs and BMFs, field BAFs and field TMFs. B metrics relevant to air-breathing animals (e.g., field BMFs/TMFs, terminal elimination half-lives) can also be considered. For each LOE, the BAT includes Data Evaluation Templates (DETs) developed from standardized testing guidance to evaluate the reliability of the LOE used in the assessment. In vivo, in vitro and in silico biotransformation rate data can be entered to predict various B metrics using built in mechanistic mass balance bioaccumulation models for representative vertebrate species. These in silico B metrics can then be compared to the empirical data entered by the user or if empirical data are not available, the built-in BAT models can be used to obtain various B metrics in aquatic and terrestrial organisms. As illustrative examples, the BAT 2.0 was applied to assess the bioaccumulation potential of hexachlorobenzene (HCB) and ?-hexachlorocyclohexane (?-HCH) and

successfully captures key similarities and differences in the behaviour of these two chemicals in the environment. The BAT 2.0 is freely available and can readily be used by interested stakeholders from academia, industry, and the regulatory community.

3.01.P-Mo113 Assessing Bioaccumulation of Linear Volatile Methylsiloxanes in Aquatic Species Using the Bioaccumulation Assessment Tool

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Bioaccumulation of a chemical is predicted or assessed using different metrics such as bioconcentration factor (BCF), bioaccumulation factor (BAF), biomagnification factor (BMF), and trophic magnification factor (TMF). The metrics have different criteria for bioaccumulation according to the design of the endpoint. It is not uncommon to obtain conflicting results using the different metrics. To evaluate bioaccumulation of linear volatile methylsiloxanes (VMS), we have employed the Bioaccumulation Assessment Tool (BAT, ver2.02) that facilitates the evaluation of multiple kinds of bioaccumulation data in a quantitative weight-of-evidence (QWoE) approach. The model assesses bioaccumulation potential with consideration of the relevance and reliability of each available data point. Four selected low molecular weight linear VMS were hexamethyldisiloxane (L2), octamethyltrisiloxane (L3), decamethyltetrasiloxane (L4), and dodecamethylpentasiloxane (L5). Although they are used as chemical intermediates and can be found as ingredients in consumer products, their environmental concentrations are very low or under detection limits in different media including biota, surface water, air, and sediment. Thus, there is only laboratory-based data available. According to the existing bioaccumulation studies for linear VMS, only BCF and BMF values were available for the inputs of the BAT. This is in contrast with data-rich cyclic VMS that have both laboratory and field-based data. However, the BAT can evaluate bioaccumulation potential using QSAR predicted biotransformation rates and BCFs from the EAS-E Suite (QSARINS, IFS, and OPERA) and the EPA CompTox Chemicals Dashboard. These predictions filled the knowledge gaps due to limited field measurements. According to the measured BCFs, L2 would not be considered bioaccumulative whereas L3-L5 would be considered bioaccumulative or very bioaccumulative depending on water concentrations. However, the measured laboratory BMFs of L3 and L4 supported non-bioaccumulative behavior (BMFs of L2 and L5 have not been measured yet). The use of predicted biotransformation rates and BCFs was advantageous for assessing bioaccumulation potential of the linear VMS in the BAT. The overall evaluation will be presented with all the measured and predicted bioaccumulation metrics.

3.01.P-Mo114 Bioaccumulation Assessment of Ionisable Chemicals - When Are Experimental BCF Values Necessary?

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When investigating a substance's bioaccumulation potential within a REACH PBT/vPvB assessment, the octanol-water partition coefficient (Kow) can be used in a first screening step in the absence of an experimental bioconcentration factor (BCF) in fish. In case a substance's log Kow value is greater than 4.5, it should be considered potentially B/vB and additional data are then required to assess its bioaccumulation potential. In case of substances that are expected to ionise completely or partially within an environmentally relevant pH-range (e.g., 5 to 9), a bioaccumulation screening based solely on log Kow is not always accepted as such substances can bioaccumulate through mechanisms other than partitioning to lipids. This has led to regulatory requests to perform experimental BCF studies, even in cases where the log Kow of the neutral form is several orders of magnitude below 4.5. Nonetheless, even for ionisable substances, lipid-based partitioning is still a dominant bioaccumulation mechanism. In this poster, we present an analysis of existing experimental fish BCF data of ionisable substances in relation to the Kow values of their neutral form. The findings are discussed in view of the known bioaccumulation mechanisms of ionisable substances (e.g., binding to blood proteins) as well as existing QSAR models that estimate the aquatic bioaccumulation of ionisable substances. The added value of performing experimental BCF studies for such substances, is critically examined for different scenarios.

3.01.P-Mo115 A New BCF In Silico Model Based on the Critical Body Burden (CBB) Principle

Paul Thomas and Pascal Bichere, KREATiS, France

This fish BCF model provides a quantitative prediction of bioconcentration factor (BCF) of a test substance in fish divided by the concentration of the chemical in the surrounding medium at steady state. The model is based on an *a priori* calculation method following Critical Body Burden (CBB) principles and hydrophobicity. The model uses different Simple Linear Regressions or fixed values, selected on the basis of an initial classification regarding the chemical class. The Subcooled Liquid Water Solubility (SLWS) of the compound of interest is used as the descriptor rather than the log K_{OW} which is used in most other BCF models. The method is based on a critical body burden (CBB) concept which states that: $BCF (L/Kg) = CBB (Mol/Kg) / EC_{10} (Mol/L)$ (McCarty *et al.*, 1992) The calculation of BCF was developed from the knowledge acquired in modelling the chronic toxicity to fish for MechoA 1.1 compounds following Bauer *et al.* (2018). This endpoint has been modeled in the high-accuracy QSAR iSafeRat® using fish EC₁₀ data with an R²/Q² at circa 0.9. This model is based on experimental data obtained using 32-day exposure data to fish embryos/larvae. In the iSafeRat® fishEC₁₀ model, the relationship between the toxicity and the subcooled liquid water solubility can be mathematically described using a linear simple regression up to the point where the solubility of the compounds is so low than no chronic toxic effects are found. This model can be separated into three phases, a linear regression phase, using sub-cooled liquid water solubility as the descriptor; a plateau phase where equilibrium is not fully reached during the toxicity study and finally a phase where toxicity is greater than the solubility limit (Thomas *et al.*, 2019). These data are transposed to BCF values using the equation above as a "mirror image" of the toxicity data. Issues such as the positioning of the regressions and impact of metabolism on BCF are discussed.

3.01.P-Mo116 Sensitivity Analysis for the Input Parameters for the In Vitro In-Vivo BCF Approach

Sophia Krause¹ and Kai-Uwe Goss², (1)Helmholtz centre for environmental research - UFZ, Germany, (2)Analytical

The idea to predict *in vivo* biotransformation from *in vitro* data originated decades ago in the pharmaceutical field. Later on, the methods were adapted for fish in the environmental field and have continued to evolve over the years. A lack of agreement of *in vivo* and extrapolated *in vitro* results triggered a search for possible sources of error. Here we present results of this analysis: 1) The often used mathematical extrapolation scheme needs some correction but from a quantitative perspective this brings about little change. 2) Also there is only a marginal difference between models of different complexity (well stirred liver model versus parallel tube liver model; one-box versus multiple-compartment fish model. 3) The specific consideration of desorption and permeation kinetics in blood did also not lead to noticeably different results. 4) Uncertainties in the estimated fraction unbound (f_u) term can be relevant. Here we conclude that mechanistic composition-based algorithms are to be preferred for the prediction of f_u . In the light of this analysis we have conducted a sensitivity analysis for the *in vivo* *in vitro* extrapolation scheme with all remaining relevant input parameters. The results of this sensitivity analysis are presented in this poster. We also note an immanent problem in the IVIVE approach: the induction of enzymes for the degradation of the investigated chemical that may occur during the course of a typical BCF test (OECD 305) is not accounted for by the IVIVE approach. **Acknowledgments** This research was financially supported by CEFIC LRI (ECO 47 project) and the German Environment Agency under FKZ 3718 65 406 0.

3.01.P-Mo117 Fragrances Not Only Scent: Pre-Experimental Toxicity Testing for the Freshwater Amphipod *Hyaella azteca* Bioconcentration Test (HYBIT) With Difficult to Test Chemicals

Sabine Groß¹, Ricarda Schumacher², Carolin Moertl² and Christian Schlechtriem³, (1)RWTH Aachen University, Afghanistan, (2)Fraunhofer IME / Bioaccumulation and Animal Metabolism, Germany, (3)Fraunhofer IME, Schmallenberg, Germany Bioconcentration factors (BCF) for regulatory purposes are conventionally determined experimentally by aqueous exposure bioconcentration fish tests according to OECD test guideline (TG) 305. The *Hyaella azteca* Bioconcentration Test (HYBIT) provides a non-vertebrate alternative. An OECD TG covering this new approach is in preparation. As for the fish test, the selection of suitable concentration(s) of the test substance is required to avoid toxic effects during the uptake phase. However, information on the test substance toxicity to *Hyaella* (e.g. acute 96-h LC50 or a NOAEC & LOAEC from a chronic study) are usually missing. Therefore, a toxicity test design was developed which can help in the setting of suitable experimental conditions. The protocol involving a semi-static exposure scenario was evaluated as part of the multi-laboratory ring trial supporting the development of the new OECD TG. The present study explores the suitability of the acute toxicity test for volatile chemicals in form of fragrances. The results show that the toxicity of such difficult to test chemicals can be also tested as part of a pre-experimental approach for bioconcentration tests with *Hyaella azteca*.

3.01.P-Mo118 Life Cycle Exposure to Dietary 2,2', 4,4'-Tetrabromodiphenyl Ether (BDE-47) on Medaka Fish (*Oryzias latipes*)

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Previous studies in our laboratory, resorted to 40-day oral exposures to BDE-47 in specific developmental windows of medaka (*Oryzias latipes*) did not evidence effects on the growing or breeding period. In this new study, full life cycle (i.e. 140-day) dietary exposure to 1000 ng of BDE-47/g was performed with medaka to evaluate effects on growth and reproduction (i.e. fecundity, fertility and hatchability), and to analyze the bioaccumulated BDE-47 and transferred to offspring. The biometric analysis during the growth and maturation periods did not reveal significant effects and no biased sex ratios were found. Reproductive capacity was not affected by the presence of BDE-47 in diet. There was no evidence of effects during embryo and eleutheroembryo development from parental exposure. The analytical results revealed steady BDE-47 bioaccumulation during the growing period, which remained in males in the reproductive phase, and a decreasing tendency was noted in females. The lowering BDE-47 levels in females coincided with the detected BDE-47 levels offloaded in embryos. In the 10-day-old post-hatch larvae, the BDE-47 concentrations dropped to comparatively lower values than those detected in parents suggesting an efficient metabolic process in the eleutheroembryonic and post-eleutheroembryonic phases. This 140-day dietary approach found no BDE-47 effects on medaka growth and reproduction, or in early progeny stages despite effective bioaccumulation and maternal transfer. This work was made possible by Spanish Government Grant RTI2018-096046-B-C21 funded by the MCIN/AEI/ 10.13039/501100011033 and the ERDF.

3.01.P-Mo119 Qsar-Me Profiler a New Software for QSAR Predictions and Structural Similarity Analysis

Nicola Chirico¹, Linda Bertato² and Ester Papa², (1)Università degli Studi dell'Insubria, Italy, (2)University of Insubria, Italy In the last 20 years, our research group has developed many validated QSARs for the prediction of different endpoints, useful to support the assessment of the potential hazard and risk of heterogeneous organic chemicals. Recently our research has been focussed on the development of QSARs specific for the prediction of biotransformation and bioaccumulation related endpoints. This raised the need for a user-friendly software able to collect and simplify the application of these QSARs, for both a detailed visualization of the underlying models and to generate streamlined multiple QSARs predictions. To this end, the new software "QSAR multiple endpoints profiler" (QSAR-ME Profiler) was developed using the Java™ language, thus allowing for its execution on different platforms like, for example, Windows™, Mac OS™ and Linux. This software is user-friendly, and displays the available QSARs and the related output both in tabular and graphical forms. Furthermore, the software automatically detects chemicals similar to those entered by the user, which can be used for comparison across predictions. In particular, chemicals used

to train the models, as well as chemicals entered by the user, and those most similar detected by the software, are automatically depicted allowing for an easier comparison across similar structures. The software also automatically selects the suitable QSARs and runs, if needed, the external supporting software (e.g. PaDEL-Descriptor or Toxtree), also allowing the application of multiple models in batch, which output is displayed in user-friendly tables including both single and combined predictions. QSAR-ME Profiler further expands the concepts of the other two software recently developed by our research group, i.e. QSARINS-Chem ECO.44 and IVBP-Suite, by pooling their functions in a single package. We want to highlight that the new fundamental, additional feature, i.e. the automatic depiction and detection of chemicals, is important for better profiling chemicals of interest as well as the specific endpoints. The structural depiction function allows to display the user-entered chemicals, and to compare them by means of distance measures, with the automatically detected similar chemicals included in the QSARs training sets. QSAR-ME Profiler simplifies and streamlines the application of QSARs, which are provided in bundle, and is particularly aimed for regulatory purposes. It will be available soon at <http://dunant.dista.uninsubria.it/qsar/>.

3.01.P-Mo120 A New Data Source for PBTK Models: Physiological Measures Combined With Biomolecular Organ Composition From Different Fish Species to Improve In-Silico Assessment of Bioaccumulation and Ecotoxicity

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PBTK (physiologically based toxicokinetic) modelling is an important tool with increasing relevance for the assessment of the bioaccumulation and toxicity potential of chemicals in the aquatic environment. The significance of in-silico generated results do highly depend on the exact parameterization of physiological processes, as well as the characterisation of the matrix composition and allometric measures of the organism. Typically, bioaccumulation and toxicity studies are performed as in-life studies using fish following the respective OECD Test Guidelines (OECD 305, 203 & 210). Different fish species are suggested for testing, assuming comparable results from testing chemicals at the animals' ambient conditions. However, the list of species comprises cold and warm water fish, which implies differences in metabolism and thus in the uptake and depuration kinetics of the tested compounds. For the refinement of PBTK models, we provide a holistic data set for the warm-water species *Danio rerio* and *Pimephales promelas* (both *Cyprinidae*), *Oryzias latipes* (*Adrianichthyidae*) and *Lepomis macrochirus* (*Centrarchidae*), as well as for *Oncorhynchus mykiss* (*Salmonidae*) as a representative of cold-water fish species. The dataset comprises information on allometry (age, size, sex), physiological measurements (respiratory rates), as well as composition of the different organ matrices with respect to lipid and protein content. More specifically, for three selected species (*P. promelas*, *L. macrochirus* and *O. mykiss*) we also provide information on the contents of polar and unpolar lipids to reveal the proportion of storage and membrane lipids, as the lipid content is a strong driver for bioaccumulation of uncharged chemicals, dependent on their Log K_{ow} . In addition, the contents of the main matrix proteins collagen and albumin were quantified to yield an important data basis for the assessment of ionic chemicals, which preferably bind to these molecules and drive bioaccumulation. In general, organ-specific compositions of the assessed biomolecules were observed, which were comparable across species. Nevertheless, species-specific allometries of organs differed, which implies variations for binding and release of chemicals from matrix-biomolecules. Thus, the featured dataset will provide a sound basis for PBTK modelling and significantly improve PBTK-models for in-silico assessment.

3.02 Advances in detection, remediation, transport, fate and bioaccumulation of legacy and emerging per- and polyfluoroalkyl substances (Part I)

3.02.T-01 The Direct Total Oxidizable Precursor (dTOP) Assay As a Tool to Detect Unknown PFAS in German Rivers

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The outstanding properties of per- and polyfluoroalkyl substances (PFAS) enable them to be used in a variety of industrial processes and consumer products. The group comprises several thousand compounds, which poses a major challenge for chemical analysis. Sum parameters such as the total oxidizable precursor assay (TOP assay) are of increasing importance to cover the complexity of PFAS. To gain a more comprehensive overview on the total PFAS burden, the TOP assay was further modified to the direct TOP (dTOP) assay by directly digesting a small amount of solid sample with high amounts of oxidation agents. This has the benefit of covering also non-extractable PFAS that might be overlooked in the conventional TOP assay. In the present study, a time series of suspended particulate matter (SPM) samples taken from German Rivers for the German Environmental Specimen Bank were analyzed for known (target analysis) and unknown (dTOP assay) PFAS. The results revealed the ubiquitous distribution of PFAS in the major German Rivers. In almost all samples, the dTOP assay yielded substantially higher amounts of PFAS than the conventional target analysis, thus indicating the presence of considerable amounts of unknown precursors. Temporal trends from 2005 – 2019 demonstrated a significant decline in the levels of both, known and unknown, PFAS. The decline, however, was observed to be more extensive for the known PFAS than for the unknown PFAS, thus demonstrating that the target analysis of known PFAS overestimates the temporal decline of the total PFAS burden. Accordingly, the proportion of unknown PFAS increased over time. Furthermore, additional time trend analysis separating long chain and short chain PFCAs indicated a shift to precursors of shorter-chain PFCAs over time. The results highlight the relevance of sum parameters such as the dTOP assay for a comprehensive analysis and assessment of the total environmental burden with PFAS.

3.02.T-03 Occurrence of Legacy and Novel Fluorochemicals in Aquatic and Terrestrial Chains Around a Fluoropolymer Manufacturing Plant

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It is well known that the fluorochemical plants, where poly- and perfluoroalkyl substances (PFAS) are synthesized or used, are relevant sources of PFAS for the surrounding environment. The whole characterisation of the environmental release is hindered because information on the unknown PFAS that have been using for many years or that have recently replaced the banned PFAS (and so potentially released into the environment) is often covered by patents and industrial confidentiality. Besides, to comprehensively evaluate the environmental risks posed by an industrial activity, it is crucial to track the possible transfer of the emitted pollutants through terrestrial and aquatic trophic chains of the ecosystems neighboring the production sites. In this communication, a comprehensive study to assess the environmental PFAS contamination due to legacy and novel PFAS deriving from a fluorochemical industrial site that has been releasing large amounts of PFAS in the Po River valley (Northern Italy) over the past decades, have been carried out, analysing both abiotic (wastewater, river water and soil) and biotic (vegetable, invertebrates, fish, and wild bird eggs) matrices. Abiotic and biotic samples were collected within 5 km from the fluorochemical plant. The same matrices were collected 20 km far from the industrial site, within the plume of air emissions. Additionally, wild bird eggs from clutches laid in a rural site (RS) far from any known point source of PFAS were also collected. The occurrence of nine perfluorocarboxylic acids (from C6 to C14), four perfluorosulfonic acids (C4, C6, C8 and C10), two fluorotelomer sulfonates (6:2 and 8:2 FTS), the perfluorooctane sulfonamide (FOSA) and, some novel fluorochemicals was investigated. Quantification with analytical standard was carried out for two novel perfluoro ether carboxylic acids: C6O4 (C6HF9O6; 2,2-difluoro-2-((2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl)oxy)-acetic acid; CAS No. 1190931-41-9), and the mixture of Cl-PFPECA also known as ADV (C3F6ClO-[CF(CF3)O]_e-[CF2CF(CF3)O]_p-CF2COOH, e=0-4, p=0-4, CAS No. 329238-24-6). Other novel PFAS were only revealed, since no analytical standards were available. The analysis of the collected samples revealed high contamination of the fluorochemical plant surrounding area, raising environmental concern. The contamination is not confined to few kilometers far the industrial site but atmospheric transport of emitted PFAS probably occurs.

3.02.T-04 Perfluorinated Substances in WATER, Macrophytes and Fish From the Amazon River

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Perfluorinated alkyl substances (PFASs) are receiving an increasing worldwide attention due to their persistence, toxicity and widespread occurrence. This study provides the first wide-spread assessment of the occurrence and distribution of these contaminants in the Amazon River, the largest drainage basin in the world, including three different abiotic and biotic matrices: water, macrophytes and fish. A monitoring campaign covering more than 1500 km of the Brazilian Amazon River was carried out in November-December 2019. Thirty-nine water surface samples were taken in urban streams (Manaus, Santarem, Macapá, Belém), the dilution areas of such streams and along the Amazon river (including three of its major tributaries). In the same or close areas, 17 samples belonging to three aquatic macrophyte species (*Eichhornia crassipes*, *Eichhornia azurea* and *Pontederia rotundifolia*) were gathered and analysed separately in aerial (leaves) and submerged (stems and roots) parts. Likewise, 53 fish samples were acquired at local markets in the sampled areas. Those belonged to 14 different species including carnivorous, omnivorous, herbivorous and detritivorous feeding habits. Identification of 27 individual PFAS including carboxylates, sulfonates, phosphonic, propanoic and ethanoic acids, fluorotelomers and phosphates, and also Gen X was performed by HPLC-QqQ. In the water, 12 compounds were identified for the first time in Amazon river, being PFPeA, PFOS and PFHxS the most abundant ones, and being all short chained. *P. rotundifolia* significantly bioaccumulated more PFAS than the other two species. Specifically, short-chain PFCAs were significantly more bioaccumulated on aerial parts of this species. Significant differences were also found for different PFASs families and chain lengths between plant parts. In the fish samples, PFOS and PFOA concentrations were lower than fish reported previous studies in other basins from Europe and Asia, although concentrations of compounds such as PFHxA, PFBA, PFHPA, FPNA y PFHxS were higher than expected. Gen-X was detected and further analysis will assess the presence of non-previously detected PFAS in the Amazon River. ACKNOWLEDGEMENTS: This study has partly been funded by the National Geographic Society (EC-59809C-19), www.silentamazon.com and by the Agencia Estatal de Investigación of Spain and the European Regional Development Fund through project CTM2017-84763-C3-1-R.

3.02.T-05 PFOS Bioaccumulation and Bio-Concentration Factors in Multi-Generational Zebrafish Exposures

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Bioaccumulation and bio-concentration of per- and polyfluoroalkyl substances (PFAS) strongly influence PFAS toxicity thresholds in zebrafish (*Danio rerio*) where perfluorooctane sulfonic acid (PFOS) is among the most strongly bio-accumulative and potent PFAS chemical structures. As part of a multi-generational PFOS toxicity investigation in zebrafish, PFOS bioaccumulation and bio-concentration factor (BCF) measurements were taken over extended life-time water exposures (180d) in

parental generation (P1) and first familial generation (F1) fish from a continuous exposure to environmentally-relevant PFOS concentrations (0, 0.1, 0.6, 3.2, 20, 100 µg/L PFOS) including 5 replicates per exposure and 50 fish per replicate. At 14 and 29 days post-fertilization (dpf), P1 fish were collected from each of the 5 replicate tanks and flash frozen for tissue analysis. Both male and female fish were individually collected from 5 replicate tanks for each the P1 and F1 generation fish at 180 dpf and flash frozen. PFOS was extracted from whole-body tissues for all experimental replicates, measured by LC MS/MS, and tissue concentrations calculated based on wet weights. PFOS bioaccumulation tended to follow a power law relationship with measured PFOS exposure concentration in water where apparent steady state was reached by 14 dpf where whole-body tissue concentrations remained largely constant through 180 dpf in the P1 generation. Statistically significant increases in PFOS bioaccumulation were observed in male fish relative to females across all PFOS exposure concentrations in 180 dpf fish from both the P1 and F1 generations. PFOS was strongly bio-accumulative in zebrafish tissues with mean wet weight values ranging from 255 to 2,136 L/kg. Comparative tests of measured BCFs among PFOS exposure concentrations revealed multiple instances of significant reduction in the 20 and 100 µg/L PFOS exposures relative to the sub-ppb exposures, 0.1 and 0.6 µg/L. Similar to the observation of increased PFOS bioaccumulation in males, BCF values were significantly increased relative to females for all PFOS exposure concentrations in both the P1 and F1 generations. Overall, PFOS bio-concentrates rapidly in zebrafish tissues where apparent steady state concentrations remain stable through constant life-time exposure durations, where males ultimately have greater bio-accumulative potential. Note: Abstract contents neither constitute nor necessarily reflect DOD or US EPA policy.

3.02 Advances in detection, remediation, transport, fate and bioaccumulation of legacy and emerging per- and polyfluoroalkyl substances (Part II)

3.02.T-06 Analysis of PFAS in Environmental Samples and Materials: Different Approaches Via Fluorine K-Edge XANES Spectroscopy and Combustion Ion Chromatography

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Per- and polyfluoroalkyl substances (PFAS) have emerged over the course of the last twenty years as a global pollution issue. Altogether, there are currently more than 4700 known but only partly characterized fluorinated compounds identified by the Organization for Economic Cooperation and Development (OECD). The ongoing production of new, yet unrestricted PFAS alternatives has become a major challenge for environmental routine analytics, since the state-of-the-art method LC-MS/MS relies on structural information and availability of isotope standards of the targeted compound. Fluorine sum parameters like adsorbable organic fluorine (AOF), extractable organic fluorine (EOF) and total fluorine (TF) can be applied to survey and detect the presence of large amounts of unidentified organofluorine compounds in environmental matrices. Today fluorine sum parameters have been established as a useful supplement to classic target-analytical approaches of PFAS and were implemented for the first time as a sum value “PFAS-total” in the recently revised Drinking Water Directive (2020/2184) by the European Commission. In contrast, X-ray absorption near-edge structure (XANES) spectroscopy has been widely applied to identify low concentration of element-specific contamination without pre-treatment in environmental samples in the past. The XANES approach enables a penetration depth of approx. 1 µm (at fluorine K-edge energy; depending on the matrix) which is significantly deeper than for X-ray photoelectron spectroscopy (XPS; penetration depth approx. 10 nm). Furthermore, the method is fast, non-destructive and only simple preparation of the samples is required. In our work, we combined both F K-edge XANES spectroscopy and combustion ion chromatography (CIC) in order to present a new analytical perspective on the investigation of PFAS in environmental media and material samples.

3.02.T-07 Set up and Validation of an Hplc-Ms/Ms Targeted Method for the Biomonitoring of 30 PFASs, Including Emerging GenX, DONA, and cC6O4, in Human Plasma Samples

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Per- and polyfluoroalkyl substances (PFASs) are a group of artificial compounds characterised by a high resistance to degradation. Some of the most persistent PFASs were gradually phased out and alternatives such as HFPO-DA (or GenX), DONA (or ADONA) and cC6O4 were introduced. The aim of this work was to develop and validate an isotopic dilution LC-MS/MS method to quantify 30 PFASs in human plasma samples. The sample preparation consisted in adding the internal standards diluted in methanol to plasma to crush the proteins; the solution was then vortexed and centrifuged. A linear gradient was set up to separate the analytes and the spectrometer operated in scheduled multiple reaction monitoring. A pooled plasma was prepared by mixing several human plasma samples previously screened for low PFASs concentrations and used as blank matrix. The analytes were spiked to the blank matrix at different levels in order to obtain the solutions for the calibration curve and the quality controls. Mean R² from 0.989 to 0.999 showed a good linearity for the considered concentration ranges. The selectivity of the method was suitable for most analytes and no carry-over effect was detected. The precision of the method, calculated as mean %RSD, was good both for within-run (ranged from 2.7% to 15.7%), and between-run (from 3.0% to 17.6%). The accuracy was also verified as mean within-run accuracy (ranged from 87.9% to 113.1%) and between-run accuracy (ranged from 93.6% to 107.2%). The lower limit of quantitation (LLOQ) ranged from 0.009 to 0.078 µg/L for all compounds with the exception of PFOA, PFOS, and PFHxS (from 0.116 to 0.245 µg/L). The results of the short-term stability (1 week at autosampler temperature) ranged from 87.4% to 113.5%, while the results of the long-term stability (1 month at -20°C) ranged from 84.9% to 114.5%. The analytes were also spiked to seven different plasma samples to verify the matrix effects. Accuracy of the assay was also tested in the interlaboratory

comparisons and external quality assurance schemes (ICI-EQUAS) and in the German External Quality Assessment Scheme (G-EQUAS); the results confirmed the suitability of the method, although only for a subgroup of analytes. Finally, the method was applied to 38 plasma samples collected from the Italian general population. The analytical method developed in this work was fully validated and demonstrated to be robust and suitable for the biomonitoring of both legacy and emerging PFASs.

3.02.T-08 Thyroid Hormone Disrupting ACTivity of Polyfluorinated Alkylated Substances (PFAS) Analyzed by PFAS CALUX in WATER, Animal Feed As Well As in Farmed and Wildlife Animals

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PFAS are a complex mixture of thousands of known and mostly unknown chemicals found in water, soil and animals. Our newly developed high-throughput screening PFAS CALUX aims to capture the total thyroid hormone disrupting activity from this persistent bioaccumulating compound class. We call for an urgent change in the testing paradigm from the currently regulated few PFAS congeners to the total effects of this complex mixture of thousands of PFAS by using potency factors (REPs) and PFOA-equivalents (EQ) for both the chemical and effect-based PFAS CALUX testing results. We present a visionary study in applying these REP/PFOA-EQ concept in water, soil, feed as well as from farmed and wildlife animals with our in vitro PFAS CALUX bioassay based on thyroid-transferrin disruption (TTR-TR). The aim of this study was to establish potency factors for several PFAS and apply them on complex mixtures of PFAS in water, soil, compost, grass, spiked animal tissue as well as wildlife animals from contaminated sites used in an international interlaboratory study. All here tested 14 PFAS congeners did show full dose-response curves on the PFAS-CALUX with REP values ranging from 0,0018 (PFBA) to 2,0 (PFOS) compared to PFOA (1,0). One spiked water sample, one spiked and unspiked calf liver sample, one wild pig and one fish muscle sample (from a contaminated area), one potato, grass and feed sample were tested by PFAS CALUX for PFOA-EQ and compared to the by LC/MS/MS obtained PFOA-EQs. In case of the wild pig, calf liver spiked and potato samples both chemical and bioassay-related PFOA-EQs were in the same range of magnitude (between 130 to 1600 ng PFOA-EQ/g), while in the contaminated fish muscle and calf liver (up to 11000 ng PFOA-EQ/g) samples only a small percentage of the PFOA-EQ could be explained through the by LC/MS/MS measured 6 PFAS. This indicates a strong impact from the other expected hundreds to thousands of unknown PFAS and indicates the urgent need to implement bioassays covering the whole class of such per- and polyhalogenated compounds, such as PFAS-CALUX in the testing strategy for monitoring especially contaminated sites. The PFAS bioassay results, which represent a total effect measurement on one of the most sensitive toxic effects, namely thyroid hormone disruption, can provide an important bridge between chemical PFAS analysis, measuring only a limited number of congeners as demanded from the regulators with the expected toxic effect-based activities.

3.02.T-09 Optimizing Soil Washing for PFAS Contaminated Soil

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The number of potentially highly contaminated PFAS sites in Europe is estimated to approach 100,000. Soil washing can be used both as in-situ and ex-situ technology to remove contaminants from soil. Previously this method has been shown to be effective on various soil contaminants like PCBs, petroleum residues, heavy metals, etc. To date, there are few soil-washing facilities worldwide processing PFAS contaminated soils. To support the use of soil washing as a remediation technique, this research focuses on the optimization and development of guidelines for soil-washing of PFAS contaminated soil and the reuse of the resulting processed material. Deriving K_d values (soil-water partition coefficients) for different soil size fractions representative of the materials produced at different stages in a typical washing process is one objective of this work. The findings of this research will help to develop a 1D-box-model to evaluate and model soil washing processes for different PFAS contaminated materials. Batch tests using different soil fractions (silt to gravel) from historical contaminated soil from the use of AFFF were carried out. Clean soil spiked with 26 different PFAS was also used. K_d values were calculated. Results indicate that to minimize residual PFAS concentrations in processed soil, the separation of coarse-grained fractions and fine-grained fractions like silt and clays including Organic matter (OM) must be prioritised in the soil washing process. Back diffusion of PFAS must be considered when reintroducing the soil into the environment in order to achieve low regulatory criteria set by environmental agencies.

3.02.T-10 Foam Fractionation Coupled to Electrochemical Degradation for Treatment of Per- and Polyfluoroalkyl Substances in Contaminated Water - a Pilot Scale Study

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Per- and polyfluoroalkyl substances (PFAS) have become increasingly infamous over the past decades for their persistent, bioaccumulative and toxic properties. They are ubiquitous in the aqueous environment and exceptionally difficult to remove or degrade. Foam fractionation is a relatively easy and cheap novel water treatment method that can achieve competitive PFAS removal. It works by bubbling air through the contaminated water and separating a foam fraction from the liquid phase. The foam fraction is enriched in PFAS, whereas the effluent water is relatively PFAS-free. Further treatment of the foam in an electrochemical degradation system provides a compact, robust and effective treatment train for on-site remediation of PFAS-contaminated water. In the current study, a pilot-scale electrochemical reactor with boron-doped diamond anodes was used in batch mode to degrade PFAS in landfill leachate and groundwater. Additionally, an optimized continuous process for PFAS removal from leachate water was developed using a pilot-scale foam fractionation system. This optimized process was then used

to treat both groundwater and leachate and produce PFAS-depleted effluent and PFAS-enriched foam. The foam was subsequently treated electrochemically as well, to assess the performance of the proposed treatment scheme as compared to using either of the techniques separately. The presented work was the first to explore these two technologies as a treatment train. Moreover, to the best of our knowledge, no pilot-scale investigations using real water have been conducted prior to this research. The highest PFAS degradations reached in leachate and groundwater were 78 % and 82 %, respectively. Production of short-chain PFAS was observed during the degradation process, which indicates that the degradation follows a step-wise mechanism. In the foam fractionation process, PFAS removal reached approximately 60 %, but the removal of individual long-chain compounds exceeded 90 %. For the electrochemical degradation of the produced foam, modifications to the electrochemical reactor were required to prevent overflow of foam. These experiments are currently ongoing, and similar or higher degradation efficiencies as found for the untreated leachate and groundwater are expected. The findings will be of interest to a wide range of stakeholders who are trying to decrease their aqueous PFAS contamination to acceptable levels.

3.02 Advances in detection, remediation, transport, fate and bioaccumulation of legacy and emerging per- and polyfluoroalkyl substances (Virtual Only)

3.02.V-01 Investigating the Fate of Polyfluorinated Phosphate Diesters (diPAPs) in a Lysimeter System

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Per- and polyfluoroalkyl substances (PFAS) are a group of environmental persistent, bioaccumulative and often toxic substances. While perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are intensively studied and regulated, the environmental behaviour of other PFAS such as polyfluoroalkyl phosphate diesters (diPAPs) is not yet fully understood. As a PFAS-contaminated site in southwestern Germany shows high concentrations of diPAPs in field soil, investigating their environmental fate is necessary to assess potential risks. In this study, the behaviour of 6:2 diPAP and 8:2 diPAP was investigated in an outdoor soil system (lysimeter) under near-natural conditions. In addition, the manner in which their degradation products leach through the soil horizons carried by natural precipitation was observed. To study the fate of 6:2 diPAP and 8:2 diPAP, lysimeters were filled with PFAS-free subsoil and a top layer of soil separately spiked with both diPAPs. Leachate was collected at least monthly as available. After two years, the soil in each lysimeter was sampled in five depths. The analysis of all samples was performed by UHPLC-HRMS. In the leachate of the 6:2 diPAP-spiked soil, PFCAs of different chain lengths (C₄ to C₆) were detected with perfluoropentanoic acid (PFPeA) and perfluorohexanoic acid (PFHxA) representing the most prevalent components. At the end of the experiment the upper soil layers still contained 6:2 diPAP. In the leachate of the 8:2 diPAP-spiked soil, PFCAs with chain lengths from C₄ to C₈ were detected with PFOA as the major component. The soil samples of the 8:2 diPAP variant contained high concentrations of 8:2 diPAP and low concentrations of different PFCAs. The detection of PFCAs with chain lengths C₆ (6:2 diPAP application) and chain lengths C₈ (8:2 diPAP application) in the leachate shows the degradation of diPAPs into persistent PFCAs in the environment and a subsequent PFCA-transport into deeper soil horizons. These results provide important insights into the environmental fate of diPAPs as PFAS precursors and the behaviour of the formed PFCAs as their main degradation products. The transport of PFCAs into deeper soil horizons may also serve as a pathway for human exposure to PFAS, as groundwater is an important source of drinking water.

3.02.V-02 Long-Range Environmental Transport and Atmospheric Deposition of Legacy and Emerging Per- and Polyfluoroalkyl Substances in the Arctic

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The pristine Arctic have been impacted by numerous natural and anthropogenic factors. Increasing human activities and changing climate are subjects to the major pressure of the Arctic environment. Input of man-made pollutants from local emission and remote continental sources led the Arctic acting as ultimate sink, which can cause unexpected disturbance and risk to the Arctic ecosystems. Among the chemical contaminants, per- and polyfluoroalkyl substances (PFASs) have attracted significant attention of the scientific society because they become 'forever contaminants' in the global environment. In this work, both neutral and ionic PFASs have been investigated in different environmental matrices in the Arctic. Snow, seawater and the ship-borne air samples are collected on the research vessel R/V Polarstern during the cruises PS85 and PS114 from Bremerhaven to the East Greenland Sea in summer 2014 and 2018. Air samples were collected using a high-volume air sampler operating at a flow rate of 15 m³/h for 24- 48 hours on the upper deck of R/V Polarstern. Seawater samples are collected using both Keel in-situ Pump (KISP) and 1 liter sea water for solid-phase extraction. Neutral PFASs from PUF/XAD-2, XAD-2 columns and GF filters are extracted with dichloromethane. The Oasis Wax cartridges (6 mL) are eluted using 10mL methanol with 0.1 % ammonium hydroxide for ionic PFASs. Neutral PFCs are quantified by an Agilent 6890 gas chromatography coupled to 5973 mass spectrometer using positive chemical ionization mode (GC-MS-PCI). Ionic PFCs are analysed using liquid chromatography coupled with tandem mass spectrometers (LC-MS-MS). This study confirmed that the neutral PFAS, especially FTOHs could be efficiently transported via atmosphere to the remote areas and distribute in Arctic environment. While, oceanic current transport is the important pathway for ionic PFASs. The air to sea deposition fluxes of FTOHs, suggesting degradation and transformation of the precursors is an important source of the ionic PFASs in the remote ocean. Moreover, this work showed that ice and snow melting in summer could release the PFASs accumulated in winter to the high Arctic.

3.02 Advances in detection, remediation, transport, fate and bioaccumulation of legacy and emerging per- and polyfluoroalkyl substances (Poster)

3.02.P-We050 In Vitro and In Vivo Apical Effects of Exposure to the Emerging Per-Fluoroalkyl Substance, Perfluoro-4-Ethylcyclohexane Sulphonate

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Widespread application of poly- and per-fluoroalkyl substances (PFAS) has resulted in some isomers being ubiquitous in environmental matrices. This along with their resistance to degradation has allowed them to accumulate in wildlife and humans with the potential for toxic effects. While certain isomers of concern have been phased-out or banned, other PFAS that are emerging as alternative substances are still produced and are released into the environment. One such substance of concern is perfluoro-4-ethylcyclohexanesulphonate (PFECHS). PFECHS, which is an analogue of perfluorooctanesulphonic acid (PFOS), has recently been detected in various environmental media. However, there is little information on toxic potencies or mechanisms of action of PFECHS or other cyclic-PFAS. Research reported here elucidated the effects of PFECHS in a two-tiered approach. Tier 1 focused on *in vitro* experiments and has quantified cytotoxicity and generation of reactive oxygen species (ROS) in rainbow trout cell lines, while Tier 2 focused on *in vivo* experiments and has quantified apical effects related to the development of zebrafish embryos. Results pertaining to *in vitro* cytotoxicity and ROS generation have suggested PFECHS is not as acutely toxic as PFOS. While significantly increased mortalities and abnormalities were observed at concentrations of 150ug/L and greater *in vivo*, no significant effects were observed at environmentally relevant concentrations. Determining toxicities associated with exposure to PFECHS will not only characterize a new and emerging chemical of concern but also further elucidate molecular mechanisms associated with cyclic PFAS.

3.02.P-We051 Occurrence and Bioaccumulation of Novel PFAS in the Delaware River Estuary, USA

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With the phase-out of some legacy per- and polyfluoroalkyl substances (PFAS), new PFAS have been introduced in consumer and industrial applications. Little is known about the occurrence, biological accumulation, or potential impacts of novel PFAS. Here, we used high resolution mass spectrometry to evaluate legacy and novel PFAS in surface water, passive samplers, fish muscle, and fish liver from the Delaware River, USA. This region is home to historical and current PFAS producers and users. Samples were collected within the mainstem River and tributaries at sites located between Bristol, PA downstream to Elsinboro, NJ, spanning over 50 river miles. Targeted, suspect, and non-targeted analysis were conducted, and features identified using suspect lists and non-targeted analysis were semi-quantified using surrogate normalization, or presented as raw abundances. We identified a series of ether-based novel PFAS in surface water and fish tissue from across the sampled area, with the highest abundances adjacent to and downstream from a suspected point source in southwest New Jersey. Some of the chlorinated and hydrogenated species within this series were previously identified in soil, groundwater, and local surface water; here, we present the first information establishing the presence of these and additional chlorinated, hydrogenated, and fluorinated congeners in the wider ambient aquatic ecosystem. Some novel compounds were orders of magnitude more abundant than legacy PFAS in each sampled matrix. In white perch liver, the novel Cl-PFPECAs were an order of magnitude more bioaccumulative compared to PFOS based on bioaccumulation factor calculations using surface water and fish liver tissue from the same location. We also describe previously unidentified PFAS associated with recent changes in fluoropolymer production process; spectral evidence suggests these unique structures possess variable degree of fluorination, incorporation of ethers, and multiple acid moieties. Legacy PFAS including PFNA, PFOS, and PFOA were also identified in surface water and fish tissue, with some fish muscle concentrations exceeding human consumption advisories issued by the state of NJ. This work highlights the continuing evolution of PFAS occurrence in the environment, and underscores the importance of non-targeted methods to screen for PFAS beyond limited targeted lists.

3.02.P-We052 Assessment of the Occurrence and Distribution of Per- and Polyfluoroalkyl Substances in the River Liffey, Ireland

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Per- and polyfluoroalkyl substances (PFAS) are a large family of widely used legacy and emerging contaminants that are pervasive in aquatic habitats globally. These molecules are chemically very stable and are highly resistant to biological degradation; therefore, they belong to a class of compounds that tend to persist in the environment and potentially may present a risk to the health of humans and wildlife. In Ireland, data on the occurrence of these chemicals in freshwater ecosystems is scarce. In order to assess the threat PFAS pose for the environment and public health, this study had the objective of evaluating the occurrence, levels and distribution of fifteen PFAS, both legacy and emerging, in the Liffey river in the city of Dublin. The Liffey flows through agricultural land and into a large urban area, and the sampling points selected contained multiple diffuse and point sources. Analytes were extracted by solid phase extraction (SPE) and determined by liquid chromatography triple quadrupole mass spectrometer (LC-QqQ-MS). Target compounds included 8 perfluorocarboxylates (PFCAs) C₅-C₁₂, 7 perfluorosulfonates (PFSS) C₄-C₁₀ and 1 perfluorosulfonamide (FOSA), C₈. The quality parameters for the fifteen selected compounds presented good limits of detection (LOD) ranging, in general, from 0.56–12.6 ng/L. The method was applied to assess the occurrence of PFAS in 80 river water samples over four sampling campaigns, accounting for seasonal variation. Ten out of fifteen compounds were

consistently detected in the majority of the samples, at levels that range from 1 to 400 ng/L. The study offers an overview of the correlation between use land and PFAS occurrence in a major city.

3.02.P-We053 An Investigation Into PFAS in Artificial Turf Around Stockholm

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The objective of this work was to investigate the occurrence of per- and polyfluoroalkyl substances (PFAS) in artificial turfs around Stockholm. A list of 103 football fields located in Stockholm containing artificial turf was provided by the City of Stockholm and a stratified design was used to randomly select a limited number of locations for sampling. Following collection, samples were analysed for total fluorine (TF), extractable organic fluorine (EOF) and target PFAS. Detectable levels of total fluorine were observed in all samples, with concentrations ranging from 13-310 µg/g in backing, 8-305 µg/g in filling materials, and 20-652 µg/g in blade samples. EOF analyses revealed fluorine in 6 samples of backing (?145 ng F/g), 6 filling materials (?179 ng F/g), and 9 blade samples (?192 ng F/g), while target PFAS were only detected in 3 filling and 12 backing samples at very low concentrations (?0.782 ng F/g). Overall these findings suggest that PFAS occurring in artificial turf components are polymeric and therefore unlikely to be readily bioavailable. Nevertheless, their identities and fate require further investigation, in particular with regards to disposal.

3.02.P-We054 Source Attribution of Perfluoroalkyl Substances in Wastewater Treatment Plant Influent

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Perfluoroalkyl substances (PFAS) are widely used in many consumer and industrial products including stain repellents, non-stick food paper or pans, and firefighting foams. Perfluoroalkyl acids (PFAAs) are resistant to degradation and have adverse effects to the environment and human health. This study analyzed twenty PFAAs in fourteen wastewater treatment plants (WWTP) across Canada. Due to the ubiquitous nature of PFAAs and their resistance to degradation they can be found in both WWTP influent and effluent. Previous studies have shown that in most WWTPs, regardless of treatment type, PFAA in the effluent is higher than in the influent suggesting a production of PFAS and/or a low removal efficiency. For PFAAs this is due to the limited affinity for solids sorption and transformation of precursors. Most current studies focus on the effluent in WWTP to assess removal efficiency and the pollution and hazards of contaminants reentering the environment. However, wastewater influent could give insight to help determine sources of PFAAs. This study focused on comparing and correlating the WWTP influent and possible sources (e.g. drinking water, rainwater). There were not any observable correlations with population size and PFAAs concentrations across all WWTPs influent. The total range of PFAS was 11-440 ng L⁻¹ in the influent and 20-240 ng L⁻¹ in the effluent. A breakdown of the PFAS composition shows short chain perfluoroalkyl carboxylic acids (PFCAs) dominate WWTP influent, with perfluorobutanoic acid and perfluoropentanoic acid the dominant PFAA homologues in 78% of the influent samples.

3.02.P-We055 Diffusive Gradients in Thin-Films (DGT) Technique As Screening Tool for Per- and Polyfluoroalkyl Substances (PFAS) Contamination in Wastewater-Based Fertilizers

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Per- and polyfluoroalkyl substances (PFAS) are a large group of organofluorine surfactants used in the formulations of thousands of consumer goods, including aqueous film-forming foams (AFFF) used to suppress aviation fires in training scenarios, non-stick cookware, fast-food wrappers, water-repellent fabrics, medical equipment, and plastic and leather products. As a result of the perpetual use of PFAS containing products, effluents and sewage sludge from wastewater treatment plants (WWTPs) have been observed to be an important pathway for PFAS into the environment. In Germany, phosphorus and other nutrients from sewage sludge and wastewater should be recycled in WWTPs of cities with a large population. However, it is not clear if PFAS contamination from wastewater and sewage sludge end up in novel wastewater-based fertilizers. Normally, PFAS are analyzed using PFAS protocols typically with time-consuming extraction steps and LC-MS-MS quantification. However, for screening of PFAS contaminations in sewage sludge or wastewater-based fertilizers also passive samplers based on the Diffusive Gradients in Thin-films (DGT) technique can be used for the PFAS extraction. Afterward, combustion ion chromatography (CIC) can be applied to analyze the “total” amount of PFAS on the passive sampler. Here, we show results from the DGT method in comparison to those of the extractable organic fluorine (EOF) method for a variety of wastewater-based fertilizers. Additionally, we analyzed the adsorption of PFAS on the weak anion exchanger (WAX) based DGT passive sampler binding layer by infrared and fluorine K-edge X-ray absorption near-edge structure (XANES) spectroscopy.

3.02.P-We056 Development of a Comprehensive PFAS Monitoring Strategy for the Dutch WATER SECTOR

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Short abstract Title: Development of a comprehensive PFAS monitoring strategy for the Dutch water sector Per- and polyfluoroalkyl substances (PFAS) represent a particularly complex challenge for water authorities due to their widespread use, difficult removal, the large number of compounds that fall in this category, and the limited information about their toxicity. In addition to efforts to phase out their usage and, consequently minimize their emissions to the environment, implementing

comprehensive monitoring strategies allowing to determine their occurrence and identify less known congeners and emerging PFAS is of crucial importance for water authorities. Besides sophisticated analytical techniques, which allow covering as many PFAS as possible, comprehensive monitoring strategies need to include adequate sampling strategies to cover the broad range of potential PFAS sources which can impact water bodies. The goal of this study consisted in developing and implementing a comprehensive and tailored monitoring strategy that can be used to assess the quality of water sources across The Netherlands. For this purpose, a broad range of (aqueous) samples was collected, namely surface-, groundwater, influent and effluent of wastewater treatment plants (WWTPs) as well as passive samplers for contaminated groundwater.[FB1] Both targeted and suspect and non-target screening (NTS) analysis of ultra-short (< C4) to long-chain (up to C14) PFAS were used for the analysis of the collected samples. For NTS, various workflows to detect and, where possible, (tentatively) identify PFAS were implemented. These involve among others the use of Kendrick Mass Defect to detect the presence of homologous series of PFAS and precursors, as well as *in-silico* fragmentation for tentative structure annotation. Total oxidizable precursor assay (TOPA) was also implemented to determine the influence of precursors on the overall PFAS mass balance in collected samples. Results of this study provided a unique and comprehensive picture of PFAS contamination in water sources across the Netherlands. The obtained information can be used by water authorities to tailor, per location, their routine monitoring strategies and focus only on those compounds which are most relevant, while comprehensive monitoring can be performed occasionally or upon specific request to assess whether changes have occurred.

3.02.P-We057 Reference Materials for Per- and Polyfluoroalkyl Substances (PFAS)

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Standard Reference Materials (SRMs) are homogeneous, well-characterized materials that are used to validate measurements and improve the quality of analytical data (www.nist.gov/srm). The National Institute of Standards and Technology (NIST) has a wide range of SRMs that have values assigned for legacy organic pollutants. These SRMs can serve as target materials for method development and quality assurance and quality control (QA/QC) of chemical measurements. As a unique class of organic contaminants, per and polyfluoroalkyl substances (PFAS) present measurement challenges to the environmental analytical community that can affect the accuracy and precision of quantitative measurements. Currently, NIST has eleven different reference materials with values of PFAS measured in them; however, there are relevant gaps in the NIST library of reference materials. A major gap is a reference material of technical mixtures, specifically an aqueous film-forming foam (AFFF) material. This presentation will discuss the existing reference materials, along with the past and current efforts at NIST to produce new reference materials for PFAS.

3.02.P-We058 Distribution of Per- and Polyfluoroalkyl Substances (PFAS) in Home-Produced Food Across Flanders

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Per- and polyfluoroalkyl substances (PFAS) can enter the human food chain due to their persistence, widespread use and global distribution. Generally, food is considered as the major exposure source of PFAS to humans. While plenty of data are available on commercial food products, little information exists on the contribution of self-cultivated food to the total dietary PFAS intake. Therefore, the main goal of this study was to examine the distribution of PFAS, including both legacy and emerging compounds, in home-produced food. Secondly, potential health-risks were assessed through the consumption of home-produced food to local residents. To this end, 65 volunteers were recruited across Flanders (Belgium) from industrial, urban as well as rural residential areas. Various food items (eggs from free-ranging laying hens, fruits and vegetables) were collected from the corresponding private gardens during the summer of 2021 and were analyzed for 29 target PFAS. In total, 16 PFAS could be detected in the food matrices and large differences were observed, in terms PFAS profile and concentrations, among and within food matrices. In eggs, long-chain compounds were dominant with PFOS contributing the most to the egg PFAS profile, as concentrations were detected up to 215 ng/g wet weight of whole egg content. Egg PFOS and PFBA concentrations decreased with increasing distance from fluorochemical industry, whereas this pattern was less evident or absent for other compounds and fruits/vegetables. On the other hand, fruits and vegetables contained much lower total PFAS concentrations, compared to eggs and consisted mostly of short-chain PFCAs (PFBA and PFPeA) and long-chain PFCAs, with the latter being increasingly detected as the chain length increased (?C8). Notably, several emerging PFAS were frequently detected in fruits and vegetables, whereas this was not the case in eggs. Clearly, home-produced eggs contributed to a large extent to the total PFAS exposure compared to other home-produced food matrices. In all residential areas, currently available human health guidelines with respect to PFAS were often exceeded. This study demonstrates that the consumption of home-produced food, especially eggs, can be an important dietary exposure pathway of PFAS to humans.

3.02.P-We059 ZeroPM - Alternatives Assessment

Ian Cousins¹, Romain Figuière², Luc Miaz¹, Eleni Savvidou³, Gregory Peters⁴, Ellise Suffill⁵, Mathew White⁵, Sabine Pahl⁵, Hans Peter Arp⁶ and Sarah Hale⁷, (1)Stockholm University, Sweden, (2)Department of Environmental science, Stockholm University, Stockholm, Sweden, (3)Department of Environmental Science, Stockholm University ACES, Stockholm, Sweden, (4)Chalmers University of Technology, Sweden, (5)University of Vienna, Austria, (6)NGI, Norway, (7)Norwegian Geotechnical Institute (NGI), Norway

This work package focuses on the development and application of alternatives assessment methods both for uses of persistent and mobile (PM) substances and their treatment technologies. Per- and polyfluoroalkyl substances (PFAS) are given special attention as certain PFAS are PM substances and because PFAS are under the spotlight within the European Commission's Chemicals

Strategy for Sustainability (CSS). The essential use concept, also integral to the CSS, is further developed and applied to facilitate the rapid phaseout of some PM substances, considering technical functional and social/behavioural science approaches.

3.03 Advances in exposure modelling

3.03.T-01 PERK: An Interactive Tool to Predict Concentration of Pharmaceutical Ingredients in Different Environmental Matrices

Kishore Kumar Jagadeesan and Barbara Kasprzyk-Hordern, University of Bath, United Kingdom

Predicting an active pharmaceuticals ingredient (API) concentration in the environment using different modelling approaches is an important aspect in the assessment of their environmental risk, especially for the APIs with no or limited analytical detection methods. However, handling, validating, and incorporating different datasets including prescription or consumption data of API, API metabolism data, wastewater flow data, removal efficiency during wastewater treatment, dilution factor for the modelling is often laborious and time-consuming. The aim of this presentation is to evaluate our in-house developed R based open-source software tool, PERK, to facilitate automated modelling and reporting predicted concentration (PC) and risk quotient (RQ) of a comprehensive set of APIs derived from a wide range of therapeutic classes in different environmental matrices. Prediction accuracy (PA) i.e., ratio between the PC and MC to validate the calculated PC, can be generated with the tool. The tool provides a consistent interactive and user friendly interface in a familiar dashboard layout, enabling users to visualise predicted values and compare with their measured values. Users can download data and graphs generated using the tool in .csv or publication ready images. We have used PrAna, our in-house developed R package to get API prescription data for the PC calculations in wastewater influent, effluent, and river and compared with measured concentrations (MC) for five different catchments located in Southwest England. Our initial investigation revealed that using the API metabolism including the faecal excretion rate, provided better PA. Furthermore, this approach provides better spatial resolution as PCs are calculated using postcode level prescription data. This presentation will focus on the concept and methodology used, and the software functionalities will be demonstrated with attention to its PC, PA and RQ features.

3.03.T-02 The Full Multi: An Open Source Framework for Modelling the Transport and Fate of Nano- and Microplastics in Aquatic Systems

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In the context of the increased attention placed on plastic pollution, data on microplastics (MPs) fate and hazard potential has multiplied in recent years. However, such data is still poorly understood within a risk assessment context and needs integration so that relevant exposure indicators can be derived and sources of uncertainty and knowledge gaps identified. Mathematical models, such as multimedia mass-balance environmental fate models, can be designed to integrate the most recent findings on MPs transport and fate mechanisms, becoming extremely useful tools that can improve our understanding on the processes and parameters that govern MPs fate. Furthermore such models can provide much-needed estimates of environmental exposure concentrations, and help derive relevant exposure indicators for risk assessment. Here we present an open source flexible fate and transport modelling framework for microplastics in freshwater systems that integrates all up to date MPs fate and transport process descriptors and that can easily be parameterized for a variety of spatial and temporal scales. With this model we are able to investigate on the open questions about MPs speciation and distribution in natural systems that will provide bounding information for microplastic risk assessment.

3.03.T-03 Validating Great-Er Simulations for WWTP Born Micropollutants in a River Network With Passive Sampler Campaigns. a Case-Study From Luxembourg

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The ecotoxicological impact of WWTP born micropollutants on surface waters has entailed several European countries to implement advanced treatment on biological treatment plants. Distributed modelling of whole river networks is a good alternative to costly measurement campaigns but it also demands locally specific input data and a thorough validation. Here we show how the GREAT-ER model was implemented in Luxembourg using passive sampler results for feeding the model and validating the simulation results. The passive sampler campaigns showed that best performance is achieved when WWTP emission load data are regional and of the same season. While well-known compounds like carbamazepine, diclofenac, antibiotics or β -blockers can be reasonably predicted within a factor 2, the anti-inflammatory ibuprofen showed poor predictability which was probably related to variable nitrification in the treatment plants. In-stream concentration were often underestimated and ibuprofen proved to have a comparable impact to diclofenac on surface waters.

3.03.T-04 Modelling Organic Contaminants in Northern Ecosystems Across Time, Space and Species Using the Integrated NEM Model

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There is concern over possible effects on ecosystems and humans from exposure to persistent organic pollutants (POPs) and chemicals with similar properties. A solid understanding of the link between global emissions and resulting exposure to ecosystems is vital to enable scientifically sound mitigation strategies. Here, we introduce the integration of a modified version of the ACC-Human model into the Nested Exposure Model (NEM). The NEM model is designed to comprehensively and dynamically link global emissions with biotic exposure. We evaluate NEM's ability to predict measured PCB-153 concentrations across time, space and species in northern ecosystems. Detailed environmental data, global emissions and physicochemical properties were used to predict PCB-153 concentrations in the physical environment and biota for 1930 – 2020 (resolution 5°×5° lat/long). Predicted concentrations in biota were compared to (i) measured concentrations across time and space in Norwegian spring-spawning herring (*Clupea harengus*) and Atlantic cod (*Gadus morhua*) from large baseline studies and monitoring in Norwegian marine areas, and (ii) measured concentrations in multiple species of a marine food web in the Svalbard area. The NEM reproduced well spatial and temporal trends of PCB-153 in NSS herring and cod. Individual predictions for herring and cod were slightly too high and low, respectively, when compared to measurements. Better overall model performance for herring than for cod probably reflects that herring is more homogenous than cod in terms of diet and habitat. Detailed results, including exploration of past, present and future emission scenarios, will be presented. Predicted PCB-153 concentrations were also in good agreement with measured concentrations in an Arctic marine food web on Svalbard including zooplankton, fish and marine mammals. Considering all the uncertainties inherent in input parameters, model assumptions, and measurement data, model performance is judged good. This suggests that the NEM model succeeds in quantitatively and mechanistically linking global historical emission of PCB-153 with northern ecosystem exposure, including trends across time, space and species. NEM has potential to be expanded to other contaminants, species, and ecosystems of interest, making it a useful tool for both scientific and regulatory communities interested in understanding and protecting ecosystem and human health from legacy and emerging organic contaminants.

3.03.T-05 Uncertainty Analysis at the Catchment Scale for Higher-Tier BPR and REACH Risk Assessments

Fabienne Ericher, CEA, United Kingdom

Characterising and communicating uncertainty is an important step in the evaluation of chemicals. Due to the difficulty in validating models against field data, catchment-based scenarios are often used as benchmarks to test and compare products, using worst-case assumptions. This leads to a disconnect between the risk assessment's exposure estimates and the likely real-life exposure from actual product use, which makes it difficult to progress into higher tiers, measure uncertainty and review potential benefits from mitigation measures and alternative practices. There is a common saying, that all models are wrong but some are useful (George E. P. Box). Current regulatory catchment models are useful in providing a standard for substance and product evaluation. However, they are not useful in simulating actual product use and environmental exposure. A computer model was designed to expand from standard regulatory catchment models to a more complex framework that enables the investigation of alternative behaviours. One example of the use of the model would be to visualise potential daily variation in product use across a catchment. Some variability can be simulated in behaviours within individual buildings, the use of a Box-Muller transform for parameter values which are derived from a range of experimental results, and/or the consideration of alternative treatment options. Graphical outputs from such models can be a powerful communication tool: by allowing easy visualisation of likely variations, they help risk assessors and risk managers to develop an understanding of the potential uncertainty attached to a range of scenarios and products. This, in turn, would facilitate moving away from benchmark regulatory scenarios and towards more realistic risk assessments that better simulate the link between product use and environmental exposure.

3.03 Advances in exposure modelling (Virtual Only)

3.03.V-01 Environmental Fate Modelling of Shaped Nano- and Micromaterials

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Several fate models have recently been developed for use with nanomaterials. For instance, the SimpleBox model, prescribed under REACH to simulate environmental fate and exposure of chemicals. SimpleBox has been extended to include substances that occur in a nano- or micromaterial form: SimpleBox4nano. However, the SimpleBox4nano model, as well as others, are only able to predict the environmental fate and exposure of materials that are spherically shaped, while nano- or micromaterials can have various shapes, such as fibers, plates, films, rods or irregular shapes. Now a module was developed for SimpleBox4nano to include the transport and fate of non-spherical shaped nano- or micromaterials. The new shape module consists of a selection of routines for non-spherical materials that can replace the ones in SimpleBox4nano for spherical materials. The routines included refer to the settling behavior of nano- an micromaterials in air or water accounting for the additional drag that non-spherical shaped materials are subjected to. The shape module consists of input fields referring to the longest, intermediate, and shortest side of the material and to its density. The module then classifies the material: (i) by size as 'nanoparticle', 'nanomaterial', 'microparticle', 'micromaterial' or 'coarse' based on the inserted sizes (ii) by shape as isometric, rod, plate/disc, film/sheet rod or disc, based on the proportions of the length, height and width of the particles' of material (iii) by buoyancy, as settling or rising based on the difference in density of the solid material and its surrounding medium and (iv) by the extent to which the material is subject to drag based on the calculated Reynolds Number. Subsequently, the shape module selects the appropriate routine to calculate the terminal velocity including the corrections for drag for the specific non-spherical material. The ambition is to extend the shape module with routines to simulate more environmental fate processes that need corrections for the shape of the material, such as atmospheric scavenging and deposition, attachment with natural particles and filtration in soil or sediment. SimpleBox4nano is available at rivm.nl/SimpleBox4nano and <https://github.com/rivm-syso/SimpleBox>.

3.03.V-02 Using Hydrodynamic Modeling in Combination With Passive Sampler Measurements to Evaluate PCB Mass Balance and Sources in an Estuary

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Polychlorinated biphenyls (PCBs) are a class of legacy pollutants that drive health risk at many contaminated sediment sites. Remedial actions typically seek to remove or sequester sediments with concentrations exceeding risk-based thresholds. Successful remedy design depends on identifying the sources controlling water column and sediment concentrations, which may not be effectively captured by sediment sampling in the event of ongoing external inputs (e.g., upstream inflows, point sources like combined sewer overflows, groundwater discharges) or unidentified sediment flux hotspots. To aid such efforts and help inform decision making, we investigated a coordinated use of modeling and passive sampler measurements to help characterize dissolved PCB sources at an urban estuary, the Lower Duwamish Waterway in Seattle, USA. PCB transport in the estuary was modeled using conservative tracer simulations made with a three-dimensional hydrodynamic model, i.e., assuming tidal flushing to be the sole sink of PCBs. The model simulations were used in two modes – forward and inverse. In the forward mode, sediment-water fluxes of dissolved and colloid-bound PCBs (estimated using chemical gradients measured by passive samplers co-located across the sediment-water interface) were used as an input to the hydrodynamic model. Freely dissolved PCB concentrations in the water column calculated as a result of these fluxes were compared with water-column passive sampler measurements to assess the mass balance of PCBs within the estuary. This comparison indicated that these sediment-water fluxes were too small to explain the measured water column concentrations, suggesting that either other sediment-to-water transfer mechanisms or other inputs must be predominant. This mass balance assessment was augmented with inverse modeling to infer the source locations that would be consistent with the water column PCB measurements. The hydrodynamic model was used to simulate concentrations generated by unit tracer input at potential source locations and the source strengths that yielded the best match to the measured concentrations were calculated. The sensitivity of inferred source locations and strengths to measurement precision, density of potential sources, mixing parameters, and loss processes was assessed. Results were also propagated to a food web model to evaluate contaminant uptake by various trophic levels and evaluate hypothetical source removals.

3.03 Advances in exposure modelling (Poster)

3.03.P-Mo121 Alternative Metrics for Long-Range Atmospheric Transport Potential (LRATP)

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Several metrics have been developed to characterize the potential of chemicals to undergo long-range environmental transport (LRTP). It is common to distinguish between transport- and target-oriented metrics. An example of the former metric is the characteristic travel distance (CTD in km), which describes the potential for transport in the mobile media air and/or water with simultaneous exchange with the surface media. An example of the latter metric is the transfer efficiency (TE in %), which represents an estimate of the mass flux into a selected compartment in a target region, divided by the emission mass flux in a source region. Both metrics are calculated by the non-spatially resolved, steady-state model in The OECD Tool and were motivated by regulatory needs (Wegmann et al. 2009). The objective of this study was to develop and explore the potential utility of alternative LRATP metrics which could offer a coherent description of the overall link between emissions into air and surface media exposure in a remote region. Specifically, we propose three metrics describing the potential for atmospheric dispersal (DP), transfer to surface media (TP) and contamination of surface media (CP) that complement each other by describing different and distinct aspects of long-range atmospheric transport. We define the dispersion potential (DP) as the ratio of the tropospheric residence time of a chemical of interest, divided by the tropospheric residence time of a highly volatile inert constituent. The transfer potential (TP) quantifies the fraction of the dispersed chemical which undergoes net atmospheric deposition to surface media, whereas the contamination potential (CP) quantifies the fraction of transferred chemical that accumulates in surface media. The Stockholm Convention targets chemicals that are “likely, as a result of their long-range environmental transport, to lead to significant adverse human health and/or environmental effects, such that global action is warranted”. Because most atmospherically dispersed chemicals will need to be able to accumulate in surface media to exert such effects, the CP is designed explicitly to aid in evaluations of LRTP in this regulatory context. In contrast to earlier such metrics, such as the Arctic Contamination Potential, it can be calculated with a non-spatially resolved, steady-state model such as the OECD Tool and does not require the delineation of a specific geographic target region.

3.03.P-Mo122 ChemicalDrift: Development and Application of a Lagrangian Chemical Fate Model for the Exposure Assessment of Pollutants From Shipping Activity in the Northern Adriatic Sea

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One of the main challenges in the assessment of impact of anthropogenic stressors on marine and coastal ecosystems is to estimate the exposure to these contaminants while also keeping track of their origin, especially in the case of multiple sources of chemical pollution. In particular, Lagrangian modelling can serve as a platform to assess the main drivers of contaminant fate and predict exposure concentrations in different scenarios without losing information on their origin, thus giving fundamental data on the contribution each source has on the overall exposure of the target ecosystem. Pollutants emissions from shipping due to the use of atmospheric emission abatement technologies such as exhaust gas cleaning system (scrubbers) may significantly contribute to the exposure of organic and inorganic pollutants, especially for areas that are already subjected to multiple contamination sources of

anthropogenic origin. In this work, carried out within the European project EMERGE, we developed ChemicalDrift, a new model capable to dynamically simulate the transport of chemical substances in the marine environment while accounting for the chemical's degradation, volatilization, and partitioning between phases (e.g. between dissolved and adsorbed to colloidal and suspended particles). In detail, ChemicalDrift is built as a module of OpenDrift, the open-source Lagrangian framework developed by MET Norway for modelling the trajectories and fate of objects or substances drifting in the ocean. OpenDrift is highly flexible and can be interfaced to different formats of metocean forcing data. The model has been applied to carry out an exposure assessment for Northern Adriatic Sea of selected pollutants considering both land-based sources and discharges from shipping activities. In detail, emissions from shipping have been estimated from Automatic Identification System data and emission factors from scrubber water analysis by applying the STEAM model, while ChemicalDrift forcing data for the case study area, including ocean currents, temperature, salinity, mixed layer depth, and winds have been obtained from Copernicus Marine Services.

3.03.P-Mo123 A Global Environmental Exposure Modeling Framework for Risk Assessment of Chemicals Disposed Down-The-Drain

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Despite advancements in exposure modeling and the growth of global and local data resources, environmental exposure assessment of chemicals disposed down-the-drain (DtD), including consumer product ingredients at the global scale within a consistent framework, has been a challenge over the years. Most assessments have been applied to specific geographies, using simplistic approaches to building a spatially resolved global assessment infrastructure. Challenges such as inconsistent or scarce data, particularly for countries with high assessment needs, have further complicated the evolution of spatially resolved global exposure assessment tools. However, through strategic integration of existing global data resources and established modeling tools, a standardized framework and methodology for spatial exposure modeling can be developed for the global scale. Here we present a spatially resolved global environmental exposure modeling framework that incorporates the best-available data and modeling tools. The global hydrology network from HydroSHEDS and HydroBASINS, global river flow, detailed population estimates, and the best-available country-specific water use and wastewater treatment information were integrated with the iSTREEM® framework (<https://www.istreem.org/>) to provide a means of estimating the distribution of concentrations of a chemical disposed DtD across a river network. The framework was first developed for China and further extended to include Japan, Canada, and Mexico. Country-level case studies were generated based on chemical production volume, consumer use estimates, and chemical-specific removals at wastewater treatment. Modeling results (probabilistic and spatial) from the case studies were compared with available monitoring data from literature; results showed good agreement between modeled and measured data. The framework developed is highly adaptable to parametrize the model for countries with an abundance of data (e.g., North America) or those scarce with data (e.g., developing countries). This work highlights the practical application of the model as a ready-to-use tool for exposure assessments. The iSTREEM® model's evolution reflects recent scientific advances in DtD exposure modeling to address global challenges and needs such as assessment over broad geographies, the incorporation of probabilistic variability, spatially explicit distributions, and accessibility of this enhanced utility for end-users.

3.03.P-Mo124 Regulatory Risk Assessment of Down-The-Drain Products: Giving Catchments a Story

Fabienne Ericher, CEA, United Kingdom

Risk assessments of down-the-drain products, under REACH and the BPR, are largely reliant on the specifications of the simulated catchment, from the number and type of buildings to the collective inhabitants' product usage behaviour. This poster looks at Biocides Product Type 18 (insecticides) ERA as a case study. The current emission document (OECD series on emission scenario documents – Nb 18 – emission scenario document for insecticides, acaricides and products to control other arthropods for household and professional uses) proposes default scenarios for the assessment of potential environmental emission for the use of biocides in the residential environment. The typical pathway for biocidal substances that are applied indoor are generally down-the-drain, following a wet-cleaning event indoors. The $F_{\text{simultaneity}}$ parameter, which defines the likelihood/extent of product usage across the catchment, simplify usage to a single conservative value representing daily likelihood, which is shared across all buildings. This poster aims to compare results obtained using this standard approach, to a model that more closely reflects individual consumer behaviour, where each building would have its own story. Can a more complex representation of product use provide insights into uncertainty and on the potential impact of mitigation measures which can influence user behaviour?

3.03.P-Mo125 Exposure and Risk Assessment of Contaminants in Fertilisers - Comparison of Exposure Tools and Establishment of a Strategy for Meaningful 'Screening' Assessment

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In this project for the European Commission, Directorate General Environment, the exposure and risk of contaminants/impurities of potential concern in fertilisers needed to be assessed, to identify contaminants/impurities or fertilisers for which additional regulatory measures may be required. Exposure and risk assessment was required for the different environmental compartments, as well as for secondary poisoning and humans exposed via the environment. In principle, these could all be assessed using EUSES, however, EUSES currently has too many limitations to assess the use under consideration (direct application to agricultural soil) in terms of (1) potential scenarios, (2) relevant environmental fate processes modelled, and (3) applicability for both organics and inorganics/metals. Therefore, a thorough comparison of different available tools (e.g., EUSES, FEE tool, ECPA-LET, FOCUS models, ...) was performed to select the most suitable tool for modelling this type of use. Further, for

secondary poisoning and humans exposed via the environment, the possibility to perform manual calculations or – for metals – a combination of calculations and the use of monitoring data was considered to obtain more realistic estimates of exposure where crucial input parameters are not readily available. Finally, the issue of background concentrations and source contribution analysis was carefully considered to get a clearer idea on the magnitude of the contribution of fertiliser use to the total input/presence of the assessed contaminants/impurities in the environment. The general approach followed during the project was described in this poster, whereas one of the cases is discussed in further detail in the platform presentation “PFAS in recycled fertilisers: potential issues, identified uncertainties, and need for monitoring” submitted under the session “Poly- and perfluoroalkyl substances (PFAS): Addressing Urgent Questions in the 21st Century”. It should be noted that the views expressed in the poster are those of the contractor with the context of the service contract 070201/2019/817112/SER/ENV.B2 and according to the terms of reference associated with that contract.

3.03.P-Mo126 A Tiered Approach for Ecological Exposure Estimation Using Eas-E Suite

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Thousands of chemicals require hazard, exposure and risk assessment for ecological and human health. Measured data to inform exposure characterization are quite limited in comparison to the number of chemicals requiring evaluation. Fate and exposure models are required to address data gaps. The Risk Assessment IDentification And Ranking (RAIDAR) model combines chemical fate and transport at a regional scale with bioaccumulation in aquatic, terrestrial and agricultural food webs using toxicokinetic models. RAIDAR includes representative aquatic and terrestrial ecological receptors (e.g., plants, invertebrates, fish, herbivores, carnivores) and far-field exposure to humans. The latest version of RAIDAR includes methods to better simulate the fate, bioaccumulation and exposure for ionogenic organic chemicals (IOCs). The evaluative RAIDAR model provides the opportunity for holistic exposure- and risk-based high-throughput (priority setting) and screening-level assessments. A key obstacle to parameterize environmental fate models is an absence of reliable chemical use and emission rate data. The Chemicals in Products - Comprehensive Anthropospheric Fate Estimation (CiP-CAFE) is a life cycle chemical emissions estimation model. The RAIDAR and CiP-CAFE models are integrated in the free on-line Exposure And Safety Estimation (EAS-E) Suite platform (www.eas-e-suite.com) which can autoperparameterize the models for > 50,000 chemicals and facilitates its operation in the regulatory community. Here we conduct a tiered ecological exposure assessment for selected cyclic volatile methyl siloxanes (cVMS) as a case study using different tiers of model input parameters. The model is initially parameterized with production volumes as a surrogate for emissions data and default chemical partitioning data. The model is then parameterized using the CiP-CAFE model and other higher tiered data sources for estimating chemical partitioning. The model predictions for environmental concentrations are compared with monitoring data demonstrating how the higher tiered model input data improve the RAIDAR model performance.

3.03.P-Mo127 Applying the Multimedia Fate Model SimpleBox With Temporally Resolved Emission Data -Opportunities to Support Risk Management Decisions and Design

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SimpleBox is a screening level multimedia fate model which can be applied for deriving PECs as part of Environmental Risk Assessment, deriving a chemical footprint as well as fate factors for life cycle impact assessment. The basic implementation in these applications has mostly been using the steady state (level III) calculations. SimpleBox can also perform dynamic non steady state (level IV) calculations for defined time periods and this has recently become relevant for examining environmental dynamics of substances and materials with extremes in mobility and persistence, such as PFAS and microplastics. We present the latest approach to applying SimpleBox dynamically using R (<https://github.com/rivm-syso/SimpleBox>). This is illustrated by means of two case studies: The first applies Simplebox for assessing the cumulative stock mass in environmental compartments, which feeds into cost-effectiveness analyses of risk management measures as part of Socio Economic Analysis under REACH. The second is the probabilistic and temporal prediction of local, regional and continental PECs of substances leaching from emerging PV panel designs throughout their life cycles. We show that dynamic assessments with SimpleBox provides a better understanding of the environmental fate and distribution of chemicals in time. This can contribute to evaluating the performance of policy measures in a regulatory context, and guide technological research and development towards safer and more sustainable designs.

3.03.P-Mo128 Spatiotemporal Multimedia Modelling of Nanomaterials: A Framework for Advanced Chemical Exposure Modelling

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The emergence of new and novel chemical classes in recent decades has led to concern about the potential risks they pose to the environment. One such example is that of nanomaterials (NMs), whose ability to form thermodynamically unstable suspensions means conventional equilibrium-based exposure models cannot be used to accurately predict their exposure. To address such concerns, NM exposure science has seen significant attention, resulting in the development of tools and models that arguably place NM exposure modelling ahead of that for “conventional” chemicals. One example is a focus on the development of spatial,

dynamic, and multimedia models, with the goal of facilitating the most realistic predictions of NM exposure possible with the data available. Herein, we present the state-of-the-art in NM exposure modelling, the NanoFASE model (Nanomaterial Fate And Speciation in the Environment), a spatiotemporal multimedia model of NM fate, speciation and exposure. We demonstrate its application to nano-TiO₂ and nano-Ag in the Ebro (Spain) and Thames (UK) catchments. We discuss how its flexible spatiotemporal transport framework makes it useful for broader chemical classes than just NMs. For example, in its current form it is particularly suited to modelling other particulate matter, such as micro- and nanoplastics. It is designed to be as easy as possible to transfer to different geographical regions, utilising Europe-wide or global datasets, having comprehensive automated data processing (e.g. reprojection of spatial data), and requiring a minimal amount of calibration. It has been developed in a modular fashion, which enables extensions to be written to extend its chemical domain to contaminants with a broad range of chemistries. Importantly, it is open source and aims to adhere to FAIR principles (Findable, Accessible, Interoperable and Reproducible). These combined make the model a useful asset not just in NM exposure assessment, but as a general framework for advanced exposure assessment.

3.03.P-Mo129 Application of Multimedia Fate and Transport Models to Volatile Methylsiloxanes: A Critical Review

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We critically review generic aspects and applications of multimedia fate and transport models (MFTMs) to better understand the behavior of volatile methylsiloxanes (VMS) in the environment. MFTMs have long been employed to predict chemical behavior and exposure in the environment, along with associated ecotoxicological risk assessment, and to estimate persistence, long-range transport, and bioaccumulation potential. These models require a set of inputs such as the inherent properties of the chemicals under investigation and the characteristics of the receiving environmental systems. It is critical to make accurate estimations of these parameters to ensure that model predictions are reliable. The physico-chemical properties of VMS compounds differ markedly from those of most carbon-based organic compounds, which can have a major effect on their behavior. It is, therefore, particularly important to ensure that these properties are accurately described by MFTMs. Good agreements between modeled and measured concentrations in air, sediment, and biota indicate that our general understanding of the environmental fate of VMS is reasonable: VMS compounds are “fliers” that principally partition to the atmosphere but have low redeposition potential. They are degraded in air by reacting with OH radicals with half-lives of 3–10 days, which means that they have high characteristic travel distances but low overall persistence and low target-oriented long-range transport potential. Since they are released to water and soil via the wastewater and biosolids-to-land pathways, VMS have been detected in these compartments, where exposure can be limited by hydrolysis, volatilization, and partitioning to sediments. In soil, concentrations are reduced by volatilization and clay-catalyzed hydrolysis. In aquatic food webs, metabolism in biota tends to drive trophic dilution resulting in trophic magnification factors which are often (but not always) < 1. Three key areas where model uncertainties still need to be addressed include: (i) the strength and direction of the temperature dependence for K_{OC} which will improve confidence in model predictions of partitioning, particularly in cooler regions; (ii) the fate of atmospheric reaction products including silanols which can be deposited to surface compartments and (iii) global variations in the magnitude of emissions to wastewater which will vary with socioeconomic status and regulatory restrictions on chemical use.

3.03.P-Mo130 How Confidently Can Current Computational Models Evaluate Ecological and Human Exposure to the Myriad of Chemicals in Commerce?

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More than 350,000 chemicals and mixtures registered in national and regional chemical inventories are awaiting exposure and risk assessments. Generic mechanistic exposure models are cost-effective and user-friendly tools for this goal. Excellent examples of these models, e.g., EUSES, PROTEX, USEtox, RAIDAR(ICE), have been used to track ecological and human exposure to thousands of chemicals through multiple routes from multiple sources. In general, the applicability of these models is constrained by (i) the applicability domain of tools used for parameterization of fundamental properties, e.g., quantitative structure-activity relationships (QSARs), (ii) built-in empirical relationships generalized from experimental measurements for parameterization of the rest parameters, and (iii) assumptions adopted by model algorithms, e.g., well-mixing homogenous environmental compartments, steady state, and equilibrium partition between sub-compartments. In this presentation, we evaluate the extent to which existing generic mechanistic exposure models apply to 112,000+ chemicals registered in five comprehensive chemical inventories. In brief, using the state-of-the-art QSAR tools, we parameterize the selected models with partitioning and reaction properties to predict exposures of ecological receptors and humans to this myriad of chemicals. We systematically analyze the applicability domains of QSARs, built-in empirical relationships, and model assumptions for these chemicals, whereby we determine the chemicals in these inventories for which these models can make predictions with the highest confidence.

Preliminary results indicate that we can be confident in evaluations of exposure for only a small fraction of commercial chemicals using the current computational models. Specifically, violating the assumption of well-mixing in outdoor compartments (notably large water bodies) limits the application of generic, mechanistic exposure models to most chemicals. The applicability domain of empirical relationships limits the application of generic, mechanistic exposure models to more than half of chemicals. The combination of multiple QSAR models greatly expands the applicability domain. Reliably predicting the biodegradation half-life is the current bottleneck of QSAR applications. This presentation, for the first time, systematically informs academia and regulatory agencies on the applicability and uncertainty of the generic, mechanistic exposure models.

3.03.P-Mo131 Development and Evaluation of a Heavy Metals Speciation and Transport Model in the Multimedia Environment

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A heavy metal (HM) can exist in multiple forms (species) in the environment. For more accurate and reasonable risk assessments, it is necessary to predict the concentrations of individual species of the HM as the fate and toxicity depend on the forms. For environmental modeling purposes, HMs may be divided into three groups, i.e., Group I at a single oxidation state (Al, Co, Ni, Cu, Zn, and Pb), Group II with redox process (Cr, Mn, Fe, and Cu), and Group III involving many complex speciation processes (As, Se, and Hg). In this study, we developed a multimedia model for HMs in the Groups I and II. In the model, HMs speciation is accounted for by equilibrium and kinetic approaches. With the equilibrium approach, partitioning is calculated using a partition coefficient estimated as a function of pH, soil organic matter and/or the total HM concentration. Three forms (free ion, DOM-metal, and inorganics-metal complexes) are proposed in the dissolved phase and their distribution is estimated by V.MINTEQ. With the kinetic approach, redox processes are modeled based on the relationship between oxidation and reduction rates. The model can estimate concentrations of particulate and the three dissolved phase species (for both the oxidized and reduced HMs in Group II). The predicted concentrations in water were compared against measurements obtained in South Korea. The ratios ($C_{\text{predicted}}/C_{\text{measured}}$) based on the average total concentration in water are 0.24 (Al), 0.11 (Cd), 0.28 (Co), 0.29 (Ni), 2.67 (Pb), and 0.32 (Zn) in Group I, and 0.48 (Cr), 0.41 (Cu), 0.28 (Fe), and 0.21 (Mn) in Group II, indicating generally underestimation by the model. However, most of the predictions (99.9% (Al), 99.9% (Ni), 97.6% (Pb), and 100% (Zn) in Group I, and 97% (Cr), 99.2% (Cu), 98.4% (Fe), and 96.2% (Mn) in Group II) fell within the range of the measured concentrations. The predicted concentration fractions of the dissolved and the particulate Zn were 0.56 (± 0.27) and 0.44 (± 0.26), respectively while those of the measurements were 0.74 (± 0.24) and 0.25 (± 0.24), respectively. The estimated concentration fractions of the dissolved Cr^{3+} and Cr^{VI} , and the particulate Cr were 0.29 (± 0.04), 0.13 (± 0.12), and 0.58 (± 0.08), respectively, as compared to the measured values of 0.24 (± 0.28) for dissolved Cr^{VI} . While further evaluation is needed, these demonstrate the feasibility of the model to be used for risk assessments accounting for HMs speciation. **keywords** Heavy Metals, Multimedia Model, Speciation

3.03.P-Mo132 Incorporating Channel Geometry and Sediment Characteristics Into Predictions of In-Stream Biodegradation

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Some chemical pollutants emitted via wastewater to rivers may pose a risk for aquatic organisms. In order to quantify exposure we need to understand how quickly these pollutants dissipate from receiving environments under different conditions. In rivers, removal processes include sorption to suspended solids and sediment, photodegradation and importantly biodegradation. Each of these processes will depend on chemical-specific (intrinsic) and system-specific (extrinsic) factors. Although biodegradation can be mediated by suspended organisms, fixed biofilms are believed to be much more important. As a result, the bulk biodegradation rate constant (k) should vary with the size and shape of a river's channel and the size and sorting of its bed material because these factors affect biofilm development and how much of a chemical in solution comes into contact with the bed and banks. These geomorphic controls on biodegradation, however, have not received much attention in the literature and most in-stream exposure models do not account for them. We hypothesize k is proportional to the ratio of wetted perimeter to cross-sectional area (i.e. inversely proportional to the hydraulic radius). This means biodegradation will be faster in wide, shallow channels compared to narrow, deep channels. We also hypothesize that biodegradation will be more rapid in fine and poorly-sorted sediments compared to coarse and well-sorted sediments because the sediment surface area available for biofilm colonisation is higher, although this may be confounded by the differences in the depth of the active sediment layer in rivers with different sediment characteristics. This poster outlines a research project designed to test these hypotheses using a combination of manipulative laboratory experiments, field monitoring and numerical modelling. The lab experiments employ a low-cost annular flume, where a biofilm is cultivated and water is spiked with organic pollutants, to determine their degradation rate under different channel geometries, bed material sizes and grain size distributions. Field experiments will use dye tracing to measure in-stream degradation downstream of wastewater emission points under different channel geometries and bed materials. These observations will be used to develop empirical relationships between degradation rate constants in rivers and channel geometry and sediment characteristics in order to model in-stream biodegradation.

3.03.P-Mo133 Mechanistic Explanation of Biphasic Dissipation Kinetics by FOCUS TOXSWA

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Dissipation half-lives are determined by kinetic fitting of measured overlying water concentrations as part of sediment risk assessment. Although the resulting kinetics are commonly accepted, there is no mechanistic explanation, exemplarily for biphasic dissipation kinetics such as Double First Order in Parallel. To obtain a mechanistic understanding, the parameterized FOCUS TOXSWA model was employed creating predicted dissipation half-lives and corresponding dissipation curves. Goal of this work was to evaluate, whether models based on diffusion (Fick's laws) can deliver a mechanistic explanation for biphasic kinetics. Water-sediment studies with spiked water (OECD 219&308) were conducted with fluopyram (KfOM: 162 ml/g) and bixafen (KfOM: 2244 ml/g), in which artificial and two natural sediments were employed, respectively. To measure the dissipation, overlying waters were sampled on six sampling days within 28 days. The Toxic Substances in Surface Waters (TOXSWA) model

was used to model the behavior of the compounds in the test systems. While a 75 mm depth water body and 25 mm sediment layer were assumed in the OECD 308 studies, a 60 mm depth water body and 15 mm sediment layer were set for the OECD 219 studies. Relevant sediment parameters were derived from the experimental design. In OECD 219 water-sediment systems, predicted and fitted dissipation half-lives of bixafen yielded 1.0 and 1.3 days, respectively. Those of fluopyram equaled 3.0 and 4.2 days. Bixafen has a strong adsorption affinity promoting faster dissipation into the sediment, as compared to fluopyram with a moderate adsorption affinity. Besides the adsorption affinities, the sediment's content of organic matter as well as the height of the water layer influenced the dissipation period. Spiking the overlying water results in large concentration gradients established at the interface between overlying water and sediment. In the early phase of the studies, high mass fluxes driven by a large initial gradient promoted the dissipation. In the later phase of the studies, a certain amount of fluopyram and bixafen were adsorbed on the sediment and the gradient declined slower leading to the biphasic dissipation pattern. Biphasic dissipation can be predicted by the mechanistic TOXSWA model, which considers diffusion as relevant transport process. The extent of the water layer as well as the compounds' adsorption affinity and the contents' of organic matter determine the dissipation.

3.03.P-Mo134 Estimating the Temperature Dependence of the Octanol-Air Partition Ratio: A New ppLFER Model for ?"UOA"

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The octanol-air partition ratio (K_{OA}) describes the partitioning of a chemical between air and octanol and is often used as a surrogate to describe other partitioning phenomena in environmental and atmospheric chemistry (e.g. blood-air, atmospheric particulate matter-air, polyurethane foam-air). Because the K_{OA} is regularly applied to describe partitioning processes occurring at temperatures other than 25 °C, reliable methods for obtaining quantitative information on the temperature dependence of the K_{OA} are required. While poly-parameter linear free energy relationships (ppLFERs) have previously been shown to reliably predict the K_{OA} of organic chemicals at different temperature, the current ppLFERs for calculating the enthalpy of the phase change between octanol and the gas phase ($?H_{OA}$) were developed using a relatively small dataset and could possibly be improved. In this work we build on a recently assembled, comprehensive and curated database of measured K_{OA} values to develop a new ppLFER for the internal energy of phase change between octanol and the gas phase ($?U_{OA}$). Relying on over 200 empirical $?U_{OA}$ values and the QSAR development platform QSARINS, we explored the use of Abraham solute descriptors, PaDEL molecular descriptors and the log K_{OA} at 25 °C itself as an independent variable in multiple multilinear regression equations for $?U_{OA}$. The $?U_{OA}$ of neutral organic chemicals can be reliably predicted using only a solute's hydrogen bond acidity (A) and logarithmic hexadecane-air partition ratio (L). The latter could also be substituted with the log K_{OA} .

3.03.P-Mo135 Indoor Concentrations of Flame-Retardants in Spanish Environments: A Multimedia Modelling Approach

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Flame-retardants (FRs) are present in several consumer products (i.e.: furniture foam, electronic devices and plastics) to comply with flammability standards for household and industrial items. Therefore, FRs can migrate from the consumer products meaning a potential impact on human health. In fact, these chemicals are found in indoor environments such as air, particulate matter and dust. It has been reported that some FRs are endocrine disrupting chemicals, which can affect thyroid hormone homeostasis, neurodevelopment, behavior and reproduction. Thus, there is a need in understanding the emission, transport, sorption, and distribution of FRs. Nowadays, models constitute useful tools for elucidating and predicting the emissions, behavior and fate of semi-volatile organic compounds (SVOCs) in indoor environments. i-SVOC is a software application that implements a framework for dynamic modeling of these chemicals by covering six types of indoor compartments: air (gas phase), air (particle phase), sources, sinks (i.e., sorption by interior surfaces), contaminant barriers, and settled dust. In the present study, we determined the concentrations of two FRs, tris(chloroisopropyl)-phosphate (TCIPP) and triphenyl phosphate (TPhP), in Spanish indoor environments (schools, office and homes). A model was constructed and validated using i-SVOC software considering previously measured indoor air and dust samples for both FRs, building parameters (i.e.: room volume and ventilation) and FR sources (i.e., electronic devices). To get an overview about the presence of TCIPP and TPhP in indoor environments, four scenarios were run, considering high and low ventilation, as well as high and medium emissions factors. Modelled results have shown a good adjustment with measured FR indoor air and dust levels. Consequently, the current model may be useful in the prediction of indoor levels of these two FRs (TCIPP and TPhP) as well as their exposure in large cohort studies where measurements in indoor environment are not possible.

3.03.P-Mo136 Exposure to Chemicals From Food Contact Materials: FCCmigex, a New Resource Based on Empirical Evidence

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Food contact materials and articles (FCMs), like food packaging, are a relevant source of human exposure to xenobiotics: foodstuff comes into contact with many different FCMs during transport, processing, storage, preparation, and consumption, and

chemicals in FCMs can migrate into food. Chemicals in FCMs may also leach into the environment when food packaging is littered or landfilled. We have previously compiled an inventory of 12,285 chemicals that are associated with the manufacture of FCMs, but it is not clear if these chemicals are also relevant for human and environmental exposure. Until now, exposure assessments for food contact chemicals (FCCs) have mostly focused on a few dozen chemicals of concern, such as members of the bisphenols, the phthalates, per- and polyfluoroalkyl substances (PFAS), and heavy metals. No systematic overview of which FCCs have been shown to migrate or to be extractable from FCMs has been available. Therefore, we systematically mapped evidence of migrating and extractable FCCs by analyzing over 1200 publicly available studies meeting our study's criteria and published globally up to May 2021. The systematic analysis of the studies was carried out by trained experts applying a custom-built software tool developed by the Food Packaging Forum and partners. As a result, a freely available database (FCCmigex) provides details about approximately 3000 different FCCs with evidence for migration and/or extraction from FCMs. These data can serve as a basis for modeling exposure to chemicals from food contact materials and articles. It can support shifting the focus from a few well-known and widely tested FCCs to the entirety of FCCs with evidence for presence in food and thus relevance for human exposure. Furthermore, FCCmigex facilitates recognition of trends in chemicals use over time, which is important for exposure assessment, enables identification of key knowledge gaps as well as further systematic reviews, and provides a basis for the prioritization of migration measurements and toxicological studies. In this presentation, we will describe the methodology and process for deriving the systematic evidence map and show how these results can be used for human exposure modeling and for informing environmental exposure assessments.

3.03.P-Mo137 Probabilistic Cancer Risk Assessment to Polycyclic Aromatic Hydrocarbons (PAHs) in Human Breast Milk From Colombia

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Most polycyclic aromatic hydrocarbons (PAHs) and their derivatives are generated through anthropogenic activities. The diet is the main route that polycyclic PAHs enter the body and measuring breast milk is one of the best ways to understand the maternal body burden and can be passed on to infants. In this study, it was measured the concentrations of 23 PAHs in milk samples from 3 Colombian cities (Bogota, Cartagena and Medellin) and assessed the potential cancer risk to human. PAH exposure and risk assessment via breast milk in infants was estimated under different scenarios. Outlining Daily intake dose (DID), hazard quotient (HQ) and incremental lifetime cancer risk (ILCR) as simple point estimations are not accurate because they are obtained from measured factors containing uncertainties of a variety of sources. Therefore, we performed an uncertainty analysis associated relative errors of the factors through the Monte Carlo simulation (MCS). Consequently, the general purpose of the MCS technique was to determine the level of uncertainty of the risk model. MCS uses randomly sampling from probability distribution functions (PDF) of the random variables involved to approach the solution of a mathematical model. Monte Carlo simulations were used to estimate the hazard quotient (HQ) and incremental lifetime cancer risk (ILCR) for infant dietary exposure to PAHs. HQs were below the safe thresholds ($HQ < 1$) while ILCRs were greater than the reference value equal to 10^{-6} ($\text{mg kg}^{-1}\text{day}^{-1}$). Dietary source assessment indicated that fish is a significant source of PAHs, with mothers that consumed fish at least once per week having ~2.5 times greater PAH milk concentrations than other groups. While disparity was also observed between consumers of exclusively marine (?PAHs 198.5 ng mL⁻¹ lipid base) or freshwater fish (?PAHs 85.7 ng mL⁻¹ lipid base). However, geographical considerations can be significant in this finding.

3.03.P-Mo138 First Characterization of Blue Mussel Gene-Based Sensor in Mediterranean Ports (Corsica, France)

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Coastal port areas are subjected to a myriad of pollution sources. Contaminants in such systems include legacy pollutants (metals and hydrocarbons) and emerging contaminants (e.g. pharmaceuticals) with largely unknown effects. Monitoring for such complex chemical mixtures in marine ecosystems is difficult. Transcriptomics is a promising approach applied to toxicity monitoring in the 21st century but its development is challenging for non-model organisms and emerging contaminants. The Coastal Biosensor for Endocrine Disruption (C-BED), has been developed in Boston harbour (USA) to detect emerging contaminants and their effects on *Mytilus edulis*. The assay measures biological responses and produces a signature identifying which contaminants the mussels have been exposed to. The present study was designed to verify the use of the C-BED assay on another mussel specie, *Mytilus galloprovincialis*, in a Mediterranean ecosystem. Mussel were caged on one port area in North Corsica (St-Florent) and on one reference station (Punta Revellata). After three months of exposure, mussels were collected and dissected immediately. Gene expression analyses targeting the nongenomic oestrogen signalling pathway and steroidogenesis pathway were performed on digestive gland tissue. In parallel to these analyses, the stress response (*via* superoxide dismutase [SOD], catalase [CAT] and glutathione peroxidase [GPx] activity) of mussel and the bioaccumulation of trace element in their tissue were measured. The first results demonstrated that the trace elements contamination is higher in St-Florent compared to the reference station. This is reflected in the stress response of mussel, as CAT and GPx activities were also higher on contaminated site. The results from the C-BED assay indicate that mussels at St-Florent may be experiencing endocrine disruption. The C-BED assay provides an innovative method to monitor for chemicals and expands our ability to detect emerging contaminants before they cause ecological damage or public health concerns. It will help the local authorities to the progress of port clean-ups and evaluate water quality, aiming to promote and integrated management of these port zones.

3.04 Advances in the detection, monitoring and fate of emerging contaminants in the environment (Part I)

3.04.T-01 Three Reasons Why the Water Framework Directive (WFD) Fails to Identify Pesticide Risks

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The Water Framework Directive (WFD) demands that good status is to be achieved for all European water bodies. While governmental monitoring under the WFD mostly concludes a good status with respect to pesticide pollution, numerous scientific studies have demonstrated widespread negative ecological impacts of pesticide exposure in surface waters. To identify reasons for this discrepancy, we analysed pesticide concentrations measured in a monitoring campaign of 91 agricultural streams in 2018 and 2019 using methods that go beyond the requirements of the WFD. This included a sampling strategy that takes into account the periodic occurrence of pesticides and a different analyte spectrum designed to reflect current pesticide use. We found that regulatory acceptable concentrations (RACs) were exceeded for 39 different pesticides at 81% of monitoring sites. In comparison, WFD-compliant monitoring of the same sites would have detected only eleven pesticides as exceeding the WFD-based environmental quality standards (EQS) at 35% of monitoring sites. We suggest three reasons for this underestimation of pesticide risk under the WFD-compliant monitoring: (1) The sampling approach - the timing and site selection are unable to adequately capture the periodic occurrence of pesticides and investigate surface waters particularly susceptible to pesticide risks; (2) the measuring method - a too narrow analyte spectrum (6% of pesticides currently approved in Germany) and insufficient analytical capacities result in risk drivers being overlooked; (3) the assessment method for measured concentrations - the protectivity and availability of regulatory thresholds are not sufficient to ensure a good ecological status. We therefore propose practical and legal refinements to improve the WFD's monitoring and assessment strategy in order to gain a more realistic picture of pesticide surface water pollution. This will enable more rapid identification of risk drivers and suitable risk management measures to ultimately improve the status of European surface waters.

3.04.T-02 Large-Scale Chemical Monitoring of the Aquatic Environment: Perspectives From the Largest Global Study

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Significant knowledge gaps exist regarding the occurrence of Active Pharmaceutical Ingredients (APIs) in the environment, particularly regarding the socioeconomic indices potentially driving contamination. Available data focuses on a heavily studied set of relatively few APIs and studies often overlook potential toxicity/risk when presenting monitoring data. Here, we present new findings from the largest global study of APIs in the aquatic environment. Novel sample collection methodologies were used to characterise API contamination in >250 rivers of 104 countries of all continents, thus representing the pharmaceutical fingerprint of 471.4 million people. Of these countries, 36 had never been monitored previously for APIs. Duplicate grab samples were obtained from 1052 locations via 137 sampling campaigns using identical water collection/storage methodologies. All samples were sent frozen to the University of York and analysed for 61 APIs using a single validated analytical method. Globally, 53 of the 61 APIs were quantified. Highest concentrations were observed at locations associated with (a) inputs from pharmaceutical manufacturing, (b) untreated sewage, (c) arid climates and (d) sewage exhauster truck and municipal waste dumping. Available predicted no effect concentrations and critical environmental concentrations for the studied APIs indicate concentrations are typically lower than those that may elicit ecological effects. The exceptions were amitriptyline, citalopram, fexofenadine, ketotifen, loratadine, propranolol, sulfamethoxazole and verapamil. River concentrations exceeded safe effect levels for at least one API at 270 of 1052 study sites. Distance-based linear modelling indicates API concentrations were positively associated with population, median age, unemployment, and poverty rates (AICc=325.26, p=0.025, cumulative r²=0.241). Furthermore, multicollinearity analysis indicates the colinear indicators of the most significant socioeconomic factor associated with API contamination (population) included disability-adjusted life years attributable to the environment (r=0.95), real gross domestic product (r=0.74) and the amount of produced, collected and untreated municipal wastewater (r=0.66-0.69). As data availability and analytical techniques improve, novel monitoring approaches for emerging contaminants in the environment should include reference to both toxicity and socioeconomics to better understand where/how future monitoring may be most beneficial.

3.04.T-03 Pharmaceuticals in Sewage Sludge: Degradation Studies

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Pharmaceutical residues are always present in sewage sludge and this is among major global problems. In recent years studies have been conducted with the aim of optimizing sewage sludge composting technologies directed to the efficient degradation of pharmaceutical residues present in this matter. Without reliable treatment, sewage sludge and its compost pose a threat to human health due to the presence of a large number of different persistent organic pollutants in these matters. Peat is widely used as a bulking agent in sewage sludge compost by wastewater treatment companies in Estonia. It is preferred due to peat's ability to provide better aeration in compost piles. Due to this phenomenon, the current work was devoted to finding ways of speeding up the sewage sludge composting process in the sewage sludge mixtures with peat. The degradation rate of the studied pharmaceuticals in these mixtures – diclofenac, triclosan, and carbamazepine – turned out to be poor or even negligible. This phenomenon was essential as the C/N ratio of the sewage sludge and peat mixture was only 10 at the beginning of the experiment, and during a 3-months period remained unchanged. It is known from previous studies that triclosan and especially carbamazepine are persistent during different sewage sludge treatments, but diclofenac does not suffer from this drawback. Peat affects the

microbial population by its antimicrobial properties. Based on the experiments performed, it can be concluded that the residues of pharmaceuticals did not affect the microbial population, but other factors like pH, macronutrient content, and C/N ratio were limiting the microbial communities' activity and biomass. Sawdust turned out to be the best bulking agent in the sewage sludge compost mixture, assuring a reasonable duration of the maturation period of compost and sufficient degradation level of several widely used pharmaceuticals. Still, some of the studied residues of pharmaceuticals, as for example carbamazepine, and also triclosan, did not show full degradation or even any degradation during different composting periods. It is of utmost importance to find ways of eliminating persistent organic pollutants from sewage sludge compost. The work should be continued in developing more efficient composting technologies. Both novel compost mixtures should be developed, and the fate of different pollutants should be studied.

3.04.T-04 Increasing Trends of Legacy and Emerging Organic Contaminants in a Dated Sediment Core From East-Africa

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Temporal trends in levels of industrial organic contaminants can reflect how environmental burdens respond to changes in production, regulation, and other anthropogenic and environmental factors. There are numerous studies documenting such trends from the Northern Hemisphere, while there is very limited data in the literature from sub-Saharan Africa, where growth in population and consumption has been high, and there is low capacity for environmentally sound waste and e-waste handling. We hypothesized that the temporal trends of legacy and contemporary industrial contaminants in sub-Saharan Africa differ greatly from the Northern regions in which many of these chemicals were initially produced and more extensively used. To investigate this, a dated sediment core covering six decades from a floodplain system in urban Dar es Salaam, Tanzania, was analysed. Layers of the sediment core were analysed for the persistent organic pollutants (POPs) polychlorinated biphenyls (PCBs) and polybrominated biphenyl ethers (PBDEs), and the chemicals of emerging concern (CECs) alternative brominated flame retardants (aBFRs), chlorinated paraffins (CPs), and dechloranes using gas chromatography-high resolution mass spectrometry. All groups of chemicals showed a steep increase in concentrations towards the uppermost sediment layers reflecting the more recent years. This trend was particularly notable for CPs and the aBFR decabromodiphenyl ethane (DBDPE). Concentrations of the individual compound groups in surface sediment were found in the order CPs >> aBFRs ~ PBDEs > dechloranes ~ PCBs. Time trends for the individual compounds and compound groups differed, with PCBs being present in sediments since at least the early 1960s, while DBDPE first occurred in sediments corresponding to the last decade. The POPs time trends corresponded to trends from other developing regions but diverged from Northern industrialized regions. For CECs, trends were generally consistent with both developing and industrialized regions. Investigations into potential drivers for the observed trends showed that socioeconomic factors related to growth in the Dar es Salaam population, economy, and waste generation have contributed to the increasing concentrations of PBDEs, aBFRs, CPs, and Dechlorane Plus. Given the notable increase in POPs and CECs, further monitoring of temporal trends of these industrial organic contaminants in urban areas in the Global South is recommended.

3.04.T-05 Human Impacts on the Contamination of Canadian Lakes

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Trace organic contaminants (TrOCs) are mostly studied in drinking waters, rivers, and wastewater, but data is lacking regarding their presence in lakes. For that reason, a multi-residue method was developed to quantify 51 TrOCs in lake water. This method was applied to evaluate the contamination of 295 lakes sampled across Canada in a stratified random design with ecozones, size, and human activities in their watersheds as strata. This includes many remote lakes with limited access. The purpose of this work was to fill the gap of knowledge on lakes' contamination in Canada and to highlight the factors influencing the spatial distribution of the various contaminants. The TrOCs analyzed in this study include regulated organic pollutants, such as pesticides, as well as contaminants of emerging concern, such as pharmaceuticals, personal care products and consumer products additives. The compounds were extracted by solid phase extraction (SPE) and analyzed using ultra performance liquid chromatography coupled to triple quadrupole mass spectrometry (UPLC-QqQMS). Detected concentrations spanned from 0.4 ng L⁻¹ to 18 µg L⁻¹ and 7 TrOCs from various sources had concentrations over 1 µg L⁻¹. The most frequently encountered compound was diethyltoluamide (DEET), and at least one contaminant was detected in 84% of the analysed lakes, with 41% having a total contaminant concentration over 100 ng L⁻¹. Preliminary study of the explanatory factors of occurrence of contaminants shows that some contaminants found in the samples were linked to the type of human activity on the watersheds. For example, pesticide detection, more specifically for 2,4-dichlorophenoxyacetic acid (2,4-D), was linked to agricultural land use, while pharmaceuticals and additives, such as carbamazepine and the anticorrosive 5-methyl-1H-benzotriazole, were observed mostly in urbanized watersheds. The concentration of contaminants was also linked to the extent of land use on the lakes' watersheds. For example, pesticide concentrations increased with the amount of agriculture around a lake. The results of this work represent the first reference point to monitor the evolution of the contamination of Canadian lakes by TrOCs and demonstrate that a high proportion of the sampled lakes show a significant anthropogenic chemical footprint. More specific factors are being examined to explain the occurrence of each contaminant class in lake water.

3.04 Advances in the detection, monitoring and fate of emerging contaminants in the environment (Part II)

3.04.T-06 Field-Based Distribution and Bioaccumulation Factors for Perfluoro-4-Ethylcyclohexanesulfonate (PFECHS) in a Sedentary Waterbird Population

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The field-based distribution and bioaccumulation factor (BAF) for per- and polyfluoroalkyl substances (PFASs), including the novel cyclic perfluoro-4-ethylcyclohexanesulfonate (PFECHS, 67584-42-3; C₈HF₁₅SO₃), were determined in residential Black Swans (*Cygnus atratus*) from an urban lake (Melbourne, Australia). The concentrations of 46 PFASs were determined by HPLC-MS/MS in serum and excrement from swans, and water, sediment, aquatic macrophytes, soil and grass samples in and around the lake. Average concentrations of Σ_{46} PFASs were 120 ng mL⁻¹ in serum and 110 ng g⁻¹ dw in swan excrement, with environmental concentrations consistent with a highly impacted ecosystem. Elevated PFECHS concentrations were detected in water (27 ng L⁻¹) and serum (16 ng mL⁻¹). In the absence of credible putative alternative sources of PFECHS input to the lake, we propose that the use of high-performance vehicles are the most likely source of contamination to this ecosystem. The BAF of PFECHS was estimated as 593 L kg⁻¹, while the BAF of PFOS was 1100 L kg⁻¹. The BAF of perfluorocarboxylic acids increased with CF₂ moiety from PFOA (15.7 L kg⁻¹) to PFDoDA (2770 L kg⁻¹), indicating that further research is warranted for long-chain PFASs, especially in underrepresented lower trophic avian species. Residential species with limited ranges in urban environments, with greater levels of PFAS contamination, can serve as useful sites for the development of robust models for the transport and bioaccumulation pathways of PFASs and other novel compounds. This allows the generation of fundamental field-based BAF for novel compounds which is necessary for assessing risks posed to wildlife and is an approach that can be adopted throughout the world.

3.04.T-07 Quantification of Toxic Tire Degradant 6ppd-Quinone in Surface Waters

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For decades, coho salmon in the North West of the US suffered from acute mortality when returning to spawn from the Pacific Ocean into Urban Surface Waters. The phenomena was unidentified and known as urban runoff mortality syndrome due to its association with surface runoffs. Recently, researchers from the University of Washington, identified the cause that is associated with chemicals leached from tires that gets into surface waters. Widely used tire-rubber stabilizer 6PPD (N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine) degrades to form 6PPD-quinone, which is toxic to juvenile coho salmon and potentially toxic to other aquatic species when present even at low ug/L levels in water. Once this compound was identified with an LC-Q/TOF, there was an urgent need to quantify its presence in surface waters globally. This work describes a fast, direct-inject analytical method for the quantitation of 6PPD-quinone in surface water using liquid chromatography/triple quadrupole mass spectrometry (LC/TQ), including sample preparation, recovery, precision, stability, and reporting limit. Chromatographic resolution was found to be an important step to achieve sufficient retention and separation from possible interferences in some real-world samples. Further, Results from real-world samples collected in Canada and the United States obtained in this study indicate that samples in most urban areas have levels of 6PPD-Quinone close to the median lethal concentration for salmon calculated sub-ug/L level.

3.04.T-08 Environmental Monitoring of Selected Tire-Derived Organic Contaminants

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Runoff from highways is a vector for the transport of potentially toxic chemicals into surface waters. In this study, selected tire-derived chemicals were monitored in surface waters of rivers adjacent to two high traffic highways in the Greater Toronto Area in Ontario, Canada. Composite samples were collected from the Don River and Highland Creek in the GTA during 5 hydrological events that occurred in the period between early October 2019 and late March 2020, as well as an event in August 2020. Grab samples were collected from these rivers during a period of low flow in August 2020, as well as during a storm event in July of 2020. Analysis was performed using ultra-high pressure liquid chromatography with high resolution mass spectrometric detection (UHPLC-HRMS) using the Thermo Scientific Orbitrap Mass Spectrometer. Hexamethoxymethylmelamine (HMMM), a cross-linker of tire material, was detected at elevated concentrations (> 1 µg/L) during rain events and 15 of HMMM's transformation products were detected at a maximum summed concentration of 18 µg/L. Environmental samples were also analyzed for the tire additive, 6PPD, and its toxic oxidation by-product, 6PPD-quinone, as well as 1,3-diphenylguanidine (DPG). In many samples collected from the Don River and Highland Creek during storm events, the estimated concentrations of 6PPD-quinone exceeded the reported LC50 of 0.8 µg/L for Coho salmon exposed to this compound. Flush dynamics and temporal trends were established for each compound. Overall, this study contributes to the growing literature indicating that potentially toxic tire-wear compounds are transported via road runoff into urban surface waters.

3.04.T-09 A Rapid Screening Method for the detection of Additives in Electronics and Plastic Consumer Products

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Plastics have become an intrinsic part of our society. Since the mass production of plastics has started, the annual world production increased to 314 million tonnes in 2014. Plastics are complex mixtures of polymers and unreactive toxic intermediates, monomers, and additives, such as pigments, dyes, fillers, antioxidants, flame retardants, UV stabilizers, surfactants, and plasticizers, all to improve or modify the performance of the product. Some additives leach during use, resulting in potential human and environmental hazards. Elevated levels of various toxic additives have been detected in house dust. Still, more research is needed to identify sources in order to understand indoor exposures and fate of these substances. In addition, recycling

may introduce these toxic additives into recycled products. These findings call for fast and improved analytical techniques to identify toxic additives in consumer products and recycled plastics to better understand indoor exposure and fate. Due to the complexity of plastics multiple extractions and cleanup steps are needed before analysis can be performed, which is laborious, costly, time-consuming, and unsuitable for high throughput. To address these limitations, we developed a fast screening method by coupling an atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) to high-resolution time-of-flight mass spectrometry (HR-TOF-MS). The AP-MALDI-TOF-MS method was applied to screen for additives in various electronics and plastic consumer products. Multiple additives including antioxidants, flame retardants, plasticizers, and UV filters have been identified. Identification of certain plastic additives in plastic children's toys may indicate that they are recycled from waste electronic and electronic equipment (WEEE).

3.04 Advances in the detection, monitoring and fate of emerging contaminants in the environment (Part III)

3.04.T-11 Suspect and Non-Target Screening of Organic Contaminants in Indoor Dust From Danish Kindergartens Using Gc-Orbitrap

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Indoor dust can contain complex mixtures of hundreds of chemicals in varying concentrations. Some chemicals, such as bisphenols, phthalates, biocides, flame retardants, parabens, and poly- and perfluoroalkyl substances, have been shown to be potentially harmful. As most people spend more than 90% of their time in indoor environments, indoor dust is an important pathway for human exposure to contaminants, in particular for toddlers and infants. This study aimed to screen indoor dust from Danish kindergarten for multiple groups of contaminants, which are known to be potentially toxic, using target and suspect analysis. Furthermore, non-target analysis based on online libraries can identify further compounds not pre-selected as suspected contaminants. Dust samples were extracted using accelerated solvent extraction with a mixture of hexane and dichloromethane and cleaned on Florisil. The extracts were analyzed on gas chromatography (GC)-Orbitrap to obtain a full scan mass spectrum. The high-resolution mass spectra (HRMS) were first deconvoluted. The peaks from HRMS were picked up based on several criteria: mass error < 5 ppm, signal-noise ratio (S/N) > 10, TIC intensity > 1000, ion overlap window > 95% and sample/blank ratio > 10. An in-house suspect library for suspect screening was built up with a total of 53 chemicals. Non-target screening was performed to identify unknown compounds in the samples by matching the NIST library and MassBank libraries. Afterward, certain filters on the HRMS data were applied to identify phthalates, polycyclic aromatic hydrocarbons (PAHs), chlorinated or brominated compounds, and aromatic nitro compounds. Twenty-six known and suspected emerging contaminants were identified in 12 dust samples from Danish kindergartens using target and suspect analysis, including ten phthalate and non-phthalate plasticizers, four flame retardants, two bisphenols, three UV filters, four plastic additives, and three parabens. Notably, many banned or restricted emerging contaminants, such as Decabromodiphenyl ether (DecaBDE, BDE-209), ethyl hexyl phthalate (DEHP), Bisphenol (BPA), and parabens, were still frequently detected. Sixteen chemicals were tentatively identified using non-target screening, including four phthalate plasticizers, two organophosphate flame retardants, four polychlorinated biphenyls (PCBs), two fragrances. In addition, three contaminants with limited information were also detected, which will need further investigation.

3.04.T-12 Emerging Contaminants in Household Chicken Eggs and Soil Around Waste Disposal Sites in Tanzania

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Tanzania is experiencing rapid economic and population growth, expanding industry, and increasing import of consumer goods and potentially also waste. Limited capacity to safely manage an increasing stream of waste represents a challenge as a potential source to environmental contamination. In addition to industrial-use legacy contaminants such as PCBs and PBDEs, chlorinated paraffins and dechloranes are also associated with various consumer products and electronic waste (e-waste) and are expected to increase in developing regions. A recent study from Tanzania shows elevated levels of chlorinated paraffins (CPs) and dechloranes in air and soil samples collected in the vicinity of landfills and e-waste handling sites, as well as increasing temporal trends of both legacy and emerging contaminants. Not much is known regarding the occurrence of these high-production, emerging contaminants in African biota, their fate in the environment, and food-web dynamics. Diet is the main uptake route for contaminants in humans, especially intake of fatty foods such as animal meat and eggs. Household chickens can represent sentinel species for local contamination as they ingest a considerable amount of soil in addition to feed and household leftovers. In the present study, sampling of soil and eggs was conducted around historical and active landfills on mainland Tanzania and Zanzibar to investigate contamination from waste in general and e-waste in particular. We assess spatial variation in occurrence of chlorinated paraffins and dechloranes and the relationship between occurrence in soil and egg. Contaminant concentrations in eggs ranged from 0.3 to 8.2 ng/g lipid wt for dechloranes, < LOD – 1730 ng/g lw for SCCPs, and < LOD – 9700 ng/g lw for MCCP. Eggs from the reference location were not significantly lower compared to eggs from the landfill locations. However, concentrations of CPs and dechloranes were highest at the sampling sites in Dar es Salaam, suggesting different sources comparing Zanzibar and the Tanzanian mainland. Limited data exist for comparison of results, but high levels of chlorinated paraffins were found in eggs collected at a former e-waste recycling site in China, exceeding levels found in the present study with up to two orders of magnitude. This was as expected as China is a major producer and consumer of these contaminants. CP concentrations and congener patterns in egg and soil did not match, suggesting specific accumulation mechanisms.

3.04.T-13 Comprehensive Two-Dimensional Gas Chromatography-Time-Of-Flight Mass Spectrometry in Conjunction With Derivatisation for the Targeted and Non-Targeted Analysis of Contaminants of Emerging Concern in Wastewater

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Within the field of environmental analysis and wastewater analysis, contaminants of emerging concern, “chemicals that are currently not regulated, but may be under scrutiny for future regulation”¹, have experienced a recent surge in interest. Amongst these compounds, fractionated analysis of wastewater extracts has shown that polar compounds are the main carriers of the toxic effects of wastewater^{2,3}. While liquid chromatography remains the most common analytical platform for the analysis of more-polar compounds, gas chromatography (GC) fills the niche of non-polar to mid-polar compounds⁴. Supplementarily, derivatisation in the context of GC, allows for the conversion of more-polar analytes to non-polar derivatives, improving their amenability to GC analysis. In addition to benefits of higher separation efficiency, lower cost of operation, large availability of reference retention indices and mass spectra for compound identification⁴, the development of two-dimensional GC (GC×GC) has greatly extended the platform’s peak capacity separational capability for more-polar compounds, using both a non-polar and polar column in tandem⁵. Within this presentation, we will first discuss the development of an analytical method using GC×GC and sample derivatisation, illustrating the benefit over analysis without derivatisation and conventional one-dimensional gas chromatography. Following this, the application of the method to a large sample set of both effluent and influent wastewater samples will be discussed, characterizing both polar and non-polar contaminants, and temporal and spatial variation between samples. 1. Dulio, V. *et al.* Emerging pollutants in the EU: 10 years of NORMAN in support of environmental policies and regulations. *Environ. Sci. Eur.* **30**, (2018). 2. Smital, T. *et al.* Assessment of toxicological profiles of the municipal wastewater effluents using chemical analyses and bioassays. *Ecotoxicol. Environ. Saf.* **74**, 844–851 (2011). 3. Hrubik, J. *et al.* Toxicological and chemical investigation of untreated municipal wastewater: Fraction- and species-specific toxicity. *Ecotoxicol. Environ. Saf.* **127**, 153–162 (2016). 4. Reemtsma, T. & Quintana, J. B. *Organic Pollutants in the Water Cycle*. (Wiley, 2006). 5. Mostafa, A., Edwards, M. & Górecki, T. Optimization aspects of comprehensive two-dimensional gas chromatography. *J. Chromatogr. A* **1255**, 38–55 (2012).

3.04.T-14 Identification of Precipitation-Related Pollution Patterns in a Large River by Non-Target Screening and Cluster Analysis

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Heavy rain events potentially lead to short-term, but extraordinary changes in the composition of mixtures of organic micropollutants (OMPs). While pollution dynamics during rain events have been studied at point sources and small rivers, less is known about the impact on OMPs mixtures in large rivers. By means of high-time resolved monitoring in combination with nontarget screening and multivariate methods, this study aimed at unravelling precipitation-related pollution patterns in large rivers and at identifying relevant sources and entry pathways relevant for rain-related pollution. Daily composite samples were collected by automatic samplers at three stations located along the German part of the Moselle River since April 2021. Based on precipitation and water level data, daily composite samples were selected for chemical analysis. Chemical analysis was performed by liquid chromatography coupled to a hybrid quadrupole time-of-flight mass spectrometer. Peak picking, alignment, blank correction and annotation of known OMPs was performed in an R based workflow including an extensive in-house spectral library. Cluster analysis on the resulting peak lists was performed using the R package ‘kmL’. Since April 2021 until November 2021, 35 rain events were selected for chemical analysis. In the daily composite samples following the extreme rain events on July 14th 2021 with a maximum amount of precipitation of 145.7 l/m³*d, the pollutant composition changed dramatically. This was reflected by three pollution patterns. By means of annotated OMPs and water level data, the individual patterns represented a) wastewater-related compounds diluted with increasing water level, b) features introduced via increased groundwater discharge and c) features likely related to direct surface runoff. While for the later, the maximum feature intensity correlated with the maximum discharge, the pattern related to groundwater input was characterized by a delay in maximum feature intensity relative to the maximum water level (i.e., kinematic wave effect). The study confirmed that rain events can also have an effect on the composition of OMPs mixtures in large rivers. To gain a deeper understanding on the dynamics and sources behind the occurrence of these patterns in large rivers, monitoring is continued and the patterns are currently further characterized, i.e., by identification of functional groups and halogenated compounds, to prioritize unknown features for identification.

3.04.T-15 Impact of a Megacity on a Tropical Estuary Assessed With a Combination of Chemical Analysis and In Vitro bioAssays

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Contaminants of Emerging Concern (CECs) are a broad range of non-regulated chemicals including pharmaceuticals and personal care products (PPCPs), plasticizers, flame retardants, etc. Even though they are considered at risk for human and environmental health, there is still an important data gap on their occurrence and fate in tropical environments and under high anthropic pressure. This study aims to assess the occurrence and spatial distribution of CECs in surface water along a tropical estuary impacted by a megacity. 26 grab samples (500 mL) were collected in duplicates on a total length of 140 km - integrating the full continuum of

the Saigon River from upstream the megacity of Ho Chi Minh (9.2 million inhabitants) to the estuary mouth in the East Sea - during the dry season of March 2020. Four additional duplicate samples were collected in the main urban canals. Quantitative chemical analysis was performed using isotope dilution high performance liquid chromatography - tandem mass spectrometry (LC-MS/MS) and targeting 259 CECs (PPCPs, pesticides, flame retardants, plasticizers - among others). In addition, six *in vitro* bioassays (Er², PR, GR and PPAR α GeneBLAzer, AhR CALUX and AREc32 gene reporter assay) were conducted to capture the effects of all the active chemicals present in the samples. Finally, iceberg modelling was carried out to link the biological equivalent concentrations (BEQs) measured in both chemical and toxicity analyses. The highest quantification frequencies and total concentrations were measured in the city center and the urban canals, reaching a maximum of 2.5 $\mu\text{g/L}$ in the river and up to 100 $\mu\text{g/L}$ in an urban canal (*Bên Nghé* canal). An attenuation was observed towards the estuary: 50 chemicals were quantified in the river mouth accounting for a total of 100 ng/L. Diverse spatial trends were underscored along the continuum for the different chemical groups (PPCPs, industrial products, pesticides) highlighting the different and complex chemical mixtures that can occur in such system. Furthermore, the activation of specific effects of the reporter gene bioassays was partly masked by the cytotoxicity burst phenomenon. No general spatial tendency was observed along the river except for the urban canals which displayed high estrogenic activity, with BEQs exceeding the effect-based trigger (EBT), and high cytotoxicity.

3.04 Advances in the detection, monitoring and fate of emerging contaminants in the environment (Part IV)

3.04.T-16 In Vitro Bioassay Monitoring in Receiving Aquatic Environments Prior to Potential Discharge of Treated Oil Sands Process-Affected WATER

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As a part of the Alberta oil sands operations, there is a zero-discharge policy for oil sands process-affected water (OSPW which means that OSPW is currently stored in tailing ponds. However, with recent developments to discharge treated OSPW into regional waterbodies, several treatment technologies are underway to sufficiently remove OSPW-derived contaminants, including *naphthenic acids*, metals, polycyclic aromatic compounds, and petroleum hydrocarbons. Hence, there is a need to assess the *potential ecotoxicological impacts* of treated OSPW in the receiving aquatic environment. For a comprehensive assessment of the cell toxicity pathways of a mixture, *in vitro* bioassays are bioanalytical effect-based monitoring tools, which complement chemical analysis in water quality assessment. In this study, the chosen toxicity pathways for OSPW-derived contaminants are (1) non-specific toxicity: *cytotoxicity*; (2) specific toxicity: *activation of xenobiotic metabolism endpoints (peroxisome proliferator-activated receptor-gamma and aryl hydrocarbon receptor) and activation of endocrine estrogen receptor*; and (3) reactive modes of action: *genotoxicity and mutagenicity*. The main goal of this study will employ *in vitro* bioassays to develop a *baseline of the biological activity in the Lower Athabasca River* (Alberta, Canada) where the treated OSPW will be discharged. Additionally, this study will compare the relevant toxicity pathways associated with OSPW and municipal wastewater treatment plants whose effluents are discharged into the same river. These results will open opportunities for future evaluations of the biological conditions in the Athabasca River to compare the conditions before and after potential discharges, and to detect the long-term exposure effects.

3.04.T-17 WATERSCAN: WATER Pollution Warning System Combining bioAssays on Passive Sampling Extracts and Real Time Mixture Toxicity detection

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There is no doubt that human activity depends on water quality and availability. Despite the efforts made in the last decades for the implementation of the Water Framework Directive, water pollution is still a main cause of water quality deterioration. The traditional monitoring, based on grab samples, is not economically affordable and interest is rising in effect-based methods. Bioassays and biosensors can determine biological responses caused by complex mixtures by measuring their combined effects. The aim of WATERSCAN project is: 1. To bring to the market an already patented biofilm-based sensor that provides detection of mixture toxicity in water, real time measures and good sensitivity. 2. To test a panel of standard bioassays exposed to environmental toxic mixtures. Calibration of the PAM sensor was performed with biofilm exposed (acute and chronic exposure) to a mixture of selected heavy metals, pesticide and pharmaceuticals based on most detected and impacting compounds in European WWTP: the bio-sensor was very sensitive as YII depression was measured at environmental concentrations; biofilm growth is affected only at very high exposure rates. The prototype, protected by a granted patent, has been tested in field, first results indicate that the device can work with minimal maintenance maintaining a high level of sensitivity and a constant water flow during several days. More field tests are needed to confirm its efficiency in detecting contamination events and to better calibrate the device.

3.04.T-18 Investigating Atmospheric Sources of Organic Contaminants to the Habitats of the Saint Lawrence Beluga Whales and Southern Resident Killer Whales in Canada

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Contaminants have been identified as one of the main threats to many marine mammals, including two of the most endangered cetacean populations in Canada, the St. Lawrence Estuary beluga whales (*Delphinapterus leucas*) and Southern Resident killer whales (*Orcinus orca*). This study seeks to identify atmospheric sources of organic contaminants to their critical habitats in the St. Lawrence estuary (Quebec, QC) and Southern Salish Sea region (British Columbia, BC). A network of passive air samplers (PASs) was established to investigate the magnitude and spatial variability in the concentrations of numerous organic contaminants in the regional atmosphere and to identify potential sources. PASs were deployed at ~50 sites in each of the two regions for a period of 5 to 9 months during 2020. Extracts were analyzed for polychlorinated biphenyls (PCBs), halogenated flame retardants (HFRs), neutral poly- and per-fluoroalkyl substances, other halogenated compounds including organochlorine pesticides and natural halogenated substances, polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs, and organophosphate esters (OPEs). Multivariate statistics were applied to a data set of sequestered amounts in units of ng sampler⁻¹ day⁻¹ to explore differences and similarities between sampling sites and between compounds. The analysis identified groups of sites with similar organic contaminant signatures. For example, the concentrations of OPEs, HFRs and PCBs were elevated at most sites along the Montreal-Quebec City corridor and in the Vancouver area, consistent with a relatively high population density and industrial history of these areas. Distinct from this “urban” signature, elevated levels of PAHs and alkyl-PAHs were found at sites near oil refineries and fuel storage depots, e.g. in Levis and Trois-Rivières in QC, and Burnaby and Port Moody in BC, but also at some remote sites in BC, for which natural sources such as forest fires might be expected. A “marine” signature, with generally low levels but elevated concentrations of γ -hexachlorocyclohexane and some natural halogenated compounds (e.g. tribromoanisole) was noted at Saturna Island, BC and at sites along the outer St. Lawrence estuary, especially on the south shore. This pattern is consistent with seawater as a source of contaminants to the atmosphere. Urban, petrochemical and marine contaminant signatures appear to be quite universal across Canada and possibly beyond.

3.04.T-19 Developing a Rapid High Throughput Test to Detect Biodegradation of Environmentally Relevant Chemicals

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Pharmaceuticals are present in the aquatic environment as a consequence of their use by society, and their effects on the biota present are of growing concern. Determination of the persistence of these pharmaceutical chemicals in aquatic systems is crucial, with standardised OECD tests, including OECD tests 301 and 309 in place to assess this. However, these tests are low throughput, do not characterise the microbial inocula, and their stringent standardisation does not always accurately reflect real, variable environmental conditions, such as temperature. We developed a high-throughput, replicable method of measuring chemical biodegradation, using inocula concentrated by tangential flow filtration and microcentrifugation, to overcome the biodegradation lottery seen in degradation studies using environmental samples with low microbial biomass. This methodology was utilised in a year-long assay, which assessed the microbial cell number, microbial cell viability, time to 50% degradation (DT₅₀) of *p*-Nitrophenol and Q10, at five timepoints. Results show that the degradation rate of *p*-Nitrophenol showed less variability, a smaller DT₅₀ (P < 0.0001), and lower Q10 (P = 0.0368) with concentrated samples, compared to un-concentrated samples. This demonstrates that microbial cell number and incubation temperature exert significant impacts on the outcomes of biodegradation and persistence tests, with the methodologies established allowing for subsequent testing on multiple environmentally relevant pharmaceutical chemicals.

3.04 Advances in the detection, monitoring and fate of emerging contaminants in the environment (Virtual Only)

3.04.V-01 A Multiresidue GC-MS Method for the Enantiomeric Determination of Psychoactive Substances in Effluents and Surface Waters

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The consumption of psychoactive substances (PAS) has increased worldwide, and current wastewater treatment processes are not able to eliminate them [1,2]. Consequently, these substances have emerged as a new class of environmental pollutants. Many PAS are chiral and available either as racemate or pure enantiomers. Determination of their enantiomeric fraction (EF) and/or metabolites in surface waters is crucial for environmental risk assessment and allow discrimination between consumption, direct disposal, and synthesis pathways [3]. Therefore, the aim of this study was to develop an indirect method by gas chromatography coupled to mass spectrometry based on derivatization using (*R*)-(?)-?-methoxy-?(trifluoromethyl) phenylacetyl chloride as chiral derivatization reagent, for enantiomeric quantification of eight PAS including amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxymethamphetamine (MDMA), norketamine (NK), buphedrone (BPD), butylone (BTL), 3,4-dimethylmethcathinone (3,4-DMMC), 3-methylmethcathinone (3-MMC), and quantification of two piperazines: 1-benzylpiperazine (1-BP) and 1-(4-methoxyphenyl)-piperazine (1,4-MPP) [4]. The method was used to evaluate the occurrence, spatial distribution, and the EF of the target PAS in Portuguese surface waters in the Great Porto region and in effluents from 2 wastewater treatment plants. Regarding surface waters, both enantiomers of AMP, and only one enantiomer of MAMP, MDMA and BPD were found although below the limit of quantification (LOQ). Effluents samples showed only one enantiomer of AMP, BPD, 3,4-DMMC and both enantiomers of MDMA though at concentrations < LOQ. MAMP was also detected in both WWTPs in range of < LOQ - 57.30 ng L⁻¹ with enantiomeric fraction (EF) ?1. This is the first multiresidue analytical method by CG-MS enrolling cathinones, amphetamines, and piperazines. The presence of illicit synthetic cathinones in Douro River estuary was

reported for the first time, along with other amphetamine derivatives showing the potential of the method to monitor the target PAS [4]. Acknowledgments: This work was supported by National Funds through the Portuguese Science Foundation, FCT, I.P., project PTDC/CTA-AMB/6686/2020.

3.04.V-02 Concentrations of Organic Contaminants in Bio-Based Fertilizer Treated Soil

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The use of bio-based fertilizer (BBF) for agricultural soil treatment can reduce the dependency on chemical fertilizer and improve sustainability by recycling nutrient-rich side-streams. However, the presence of organic contaminants in BBFs may lead to the occurrence of residues in the treated soil. Thus, the objectives of this study are to measure organic contaminants concentration and evaluate the ecological risk of BBFs application in agricultural soil. Measuring a wide range of organic contaminants using a single workflow is difficult due to the complex nature of the pollutants and the complexity of the environmental matrices. In this work, first, we optimized a simple QuEChERS-based extraction and liquid chromatography quadrupole time of flight mass spectrometry (LC-qToF) method for the simultaneous quantitative analysis of a wide range of organic contaminants, including pesticides, pharmaceuticals and per- and polyfluoroalkyl substances (PFAS) in agricultural soil. The optimized workflow was then applied to screen organic contaminants in two agricultural soils treated with BBFs. The QuEChERS technique, optimized for the determination of a wide range of organic contaminants, showed satisfactory recovery for most targeted compounds. Several contaminants were detected in soil samples treated with BBFs. In all soil samples, a further suspect screening will be performed for pollutants, such as pharmaceuticals and PFAS and the risk of different BBFs treatment will be evaluated.

3.04.V-03 Occurrence and Temperature Dependence of Atmospheric Gas-Phase Semi-Volatile Organic Pollutants in Remote High-Mountains (Pyrenees)

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Effects of global diffuse pollution caused by Persistent Organic Pollutants (POPs) have been observed in fish from remote high-mountain lakes through mRNA measurements that showed feminization effects and oxidative stress. Here, polyurethane foam passive air samplers (PUF-PAS) were used for the study of the occurrence and distribution of organophosphate esters (OPE), organochlorine compounds, and polycyclic aromatic hydrocarbons (PAH) in six high-mountain sites along an altitudinal gradient in the Catalan Pyrenees (1,619–2,453 m) between 2017 and 2020. The PUF-PAS were extracted by Soxhlet using hexane, cleaned up and fractionated by high-performance liquid chromatography (HPLC), and analysed by gas chromatography coupled to mass spectrometry (GC-MS) and tandem mass spectrometry (GC-MS/MS). Experimental and estimated expanded theoretical sampling rate uncertainties were generally below 15%, indicating an adequate precision of the PRC calibration of the PUF-PASs. Extreme wind speeds (up to 130 km h⁻¹) and particle infiltration efficiency (16–23%) had limited impact for the sampler configuration used, and PAS-derived concentrations agreed with AAS samples. Atmospheric gas-phase PAH concentrations ranged between 409 ± 208 and 764 ± 201 pg m⁻³, dominated by phenanthrene (49%) and fluorene (41%). PCB were between 10 ± 3 and 18 ± 3 pg m⁻³, and relative congener compositions were 12–20% except for PCB180 (3%). OPEs were between 16 ± 9 and 53 ± 32 pg m⁻³, dominated by tris(1-chloro-2-propyl) phosphate (TCIPP, 46%). Mean hexachlorobenzene and pentachlorobenzene concentrations ranged from 17 to 90 pg m⁻³, and from 5.3 to 69 pg m⁻³, respectively. Statistically significant increases in gas-phase concentrations with temperature were observed for more than half of the studied compounds. Phase-change enthalpies (ΔH_{AS}, KJ mol⁻¹) estimated from temperature regressions were not significantly different from laboratory-estimated values for all OPEs, benz[a]anthracene, and PCB180. This indicates a dominance secondary sources and evaporation from surfaces that reflects the less volatile nature of OPEs and high molecular mass pollutants. Snowmelt was suggested as a possible main factor driving the increase of OPE gas-phase concentrations in warmer periods. Contrarily, most lighter PAHs and PCBs showed significantly lower ΔH_{AS} that point at a larger influence of advection of air masses, with differences that increased with their volatility.

3.04.V-04 Seasonal Trends of Emerging Contaminant in Water and Sediments From WWTPs in Saskatchewan

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Emerging contaminants such as pharmaceutical drugs have been detected in waters across the globe. Most pharmaceuticals are found at trace concentrations, but the continuous use and potential accumulation of some of these compounds can lead to potential effects in aquatic organisms due to their continuous-release, high biological efficacy, and possible accumulation in aquatic organisms. Wastewater treatment plants (WWTPs) are not designed to remove pharmaceuticals, so these compounds are virtually unattained before the treated water is discharged. Consequently, management of WWTPs plays a key role in the removal and monitoring of pharmaceutical products with challenges related to the polar nature of these chemicals that facilitate their presence in different environmental compartments. Pharmaceutical concentrations can be affected by temporal variations, the flow velocity of water receptors, the sorption capacity of sediments, and other abiotic dynamics in aquatic ecosystems. Currently, most experimental approaches have not considered these dynamics to evaluate the chemical activity, bioavailability, and toxicity of pharmaceuticals. The principal aim of this research is to enhance our understanding of the environmental risks associated with pharmaceuticals as one group of emerging contaminants. To this end, the presence of a suite of representative pharmaceuticals

was measured upstream and downstream of two WWTPs located in the South Saskatchewan River basin and Wascana Creek, Saskatchewan, Canada, during three sampling campaigns (spring, summer, and fall of 2021), through both conventional and passive water sampling. According to the chemical analysis conducted, Amitriptyline with a concentration of 3.35 µg/L was the most abundant compound in comparison to the other pharmaceuticals evaluated in the water and sediment samples at the four sampling sites and across the three seasons. Generally, concentrations of analytes were higher downstream of WWTPs compared to upstream sites. The data collected from this monitoring campaign indicates widespread contamination with psychoactive pharmaceuticals, which can have marked impacts on exposed organisms.

3.04.V-06 The Effect of Sample Degradation During Long Batch Acquisitions With Non-Target Analysis

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Non-target analysis (NTA) is gaining popularity as a comprehensive tool for analysis of chemicals of concern in various matrices. One possible way to use NTA in environmental monitoring is by comparing several samples, looking for differences in their compound composition (i.e. their fingerprint), due to spatial or temporal trends. These observed differences can then inform more specific analysis, like target and suspect analysis. However, depending on the matrix and compounds of interest, degradation and transformation may take place, changing the composition of the sample. Historically, this has been researched in the context of target analysis for specific compounds. However, the full effect on NTA is not known. Degradation and / or transformation may start the moment a sample is taken and can only be slowed down by appropriate storing conditions. Usually this includes temperatures of -20 or -80 °C, however samples cannot always be immediately stored or kept at these temperatures during shipping and normal lab activities. Additionally, often not considered is the waiting time of samples in the autosampler, before injection into the instrument for chromatography. Depending on the number of samples and runtime of the chromatographic method, the time between the first and last sample injection can be up to several days. If these samples are then to be compared, wrong conclusions might be made based on degradation/transformation instead of the actual differences between the samples at the time of sampling. Our aim for this study was thus to investigate how much the composition of a wastewater sample changes within normal lab timeframes. For this we obtained a fresh wastewater sample and started data acquisition with liquid chromatography – high resolution mass spectrometry (LC-HRMS) within an hour after sampling. The sample was then rerun for 24 hours, collecting 48 datapoints. Additionally, parts of the sample were left at room temperature, in the fridge and the freezer and measured daily over the following week. Results show the decrease of the intensity of some compounds over time, while others increase in intensity or have been formed as part of the degradation process. These findings will have implications for the use of NTA when conducting temporal / spatial assessments and recommendations to acknowledge or minimise these effects are discussed.

3.04.V-07 The Effect of Toxic Elements on Antioxidants Glutathione S Transferase and Diphosphotriphosphodiaphorase of Freshwater Snail *Helisoma Duryi*: A CASE Study on a Coal Mining Area

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Coal mining is destructive by nature and produces large volumes of waste. The excavation processes bring many minerals to the surface exposing them to the weather elements such as wind, humidity and precipitation. The combination of the exposed minerals and the large quantities of water used during mining results in contamination of water bodies. The contaminants accumulate in the environment and end up in the food chain and this ultimately affects human health. The aim of the present study was to assess the impact of toxic elements emanating from coal mining activities in aquatic bodies in the vicinity of a coal mine. Water samples were collected during the wet season from 6 different sites in a coal mining area in Zimbabwe. Water samples were prepared and analysed to determine the concentration of copper, lead, zinc and cadmium. Snails were exposed to sampled water over a duration of 28 days with snails collected at 7 day intervals to determine the time dependent effects of effluent from mining activities on selected biomarkers of *Helisoma duryi*. Post mitochondrial fractions were prepared from exposed snails and used to determine glutathione s-transferase (GST) and diphosphotriphosphodiaphorase (DTD) enzyme activities. Water samples had high Zinc values of 525 ± 2.63 mg/l and 540 ± 4.86 mg/l for two sites. Inhibitions of GST and DTD enzyme activities were observed in the study for the majority of the sites. Overall, this study showed that there is a need for constant monitoring of water bodies in coal mining areas to safeguard the health of aquatic life from adverse effects of effluent from coal mining activities.

3.04 Advances in the detection, monitoring and fate of emerging contaminants in the environment (Poster)

3.04.P-Tu076 Automated Methods for Monitoring the Chemical Surface Water Quality of the River Rhine

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A good chemical status of surface water bodies is fundamental for protecting aquatic communities and drinking water resources. This is expected to become even more important owing to progressing climate change and thus more frequent times of water shortage and the need for water re-use. However, the chemical status cannot be described adequately using those monitoring routines that are currently applied. Sampling campaigns are usually performed with large time intervals and the subsequent analysis and data evaluation are completed with delay. As a consequence, it is likely that event-related or occasional pollution

inputs are not recorded and protective measures cannot be initiated. We address this issue and are constructing an experimental monitoring station in Koblenz (Germany) at the river Rhine. Via automatization it provides high-resolution data in near real-time. Different analytical instruments are already installed ranging from relatively simple sensor-based technologies to more complex mass spectrometry systems. To enable a continuous operation, water is pumped from the Rhine into the station and distributed to the different instruments with or without prior filtration. Several basic water parameters, nutrients and anions can be successfully analyzed via sensors or by ion exchange chromatography. Nutrient analyses via colorimetric methods currently run in test mode. Main and trace elements will be measured via inductively coupled plasma mass spectrometry and a liquid chromatography time-of-flight mass spectrometer is available for non-target screening of organic substances. Both mass spectrometry systems are currently being modified to allow continuous measurements. Non-target analysis is not limited to specific target substances but enables the analysis of all compounds that can be effectively separated and detected by the applied chromatographic method and mass spectrometer, respectively. Especially the evaluation of non-target data is challenging and will be performed by coupling and automation of already existing, in-house-developed routines for data processing, annotation of substances and prioritization of unknown signals. We present the current status of the monitoring station and identify optimal sampling intervals for several parameters, where concentration peaks in the time-series are captured but the number of samples to be analyzed is minimized.

3.04.P-Tu077 Oceanic Transport of Pharmaceuticals From Northern Europe to the High Arctic

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Pharmaceuticals are used in large numbers across Europe which has raised concern about their impacts in marine ecosystems. Although many pharmaceuticals undergo degradation and removal in sewage treatment systems, some compounds are released to the environment where they may persist over time. Information about the concentrations of pharmaceuticals in major rivers ending in the North Sea and the Baltic Sea has been gathered and used to estimate the annual release to the marine environment of northern Europe. The potential for oceanic transport of pharmaceuticals northwards to the European Arctic has been simulated using oceanographic models. Based on these data a transect of ten sampling stations were selected starting in the North Sea and ending in at the west coast of Spitsbergen. Sampling of large volumes of water and zooplankton was performed in water masses representing the coastal systems and open sea system of the north Atlantic currents along the transect. Pharmaceuticals were directly extracted from seawater using custom made solid phase extraction columns onboard the research vessel "Oceania". The water was sampled either from sea surface (approx. 2 m, 800 L) on sections during steaming using the ship's online sampling system or from two depths (surface and 50 m, 400 L each) at stations using Niskin water bottle. Zooplankton was collected using WP-2 180 µm mesh plankton net, collecting two types of samples, mass sample for pharmaceuticals and faunistic samples for zooplankton composition and vertical distribution in the epipelagial. Oceanographic data was recorded at each sampling station. We will present the initial simulations of pharmaceutical releases and oceanic transport as well as the pharmaceutical concentrations found in seawater and zooplankton in the marine system studied.

3.04.P-Tu078 Degradation of Micropollutants by Gamma Irradiation: Insight of By-Product Formation Explored by Suspect and Non-Target Screening Using Lc-Hrms

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Incomplete removal of micropollutants remains a challenge for wastewater treatment processes. As an alternative, a decomposition process using gamma irradiation has been proposed. The aim of the study was to get insight into the degradation of micropollutants in water using gamma irradiation, the by-product formations identified by suspect/non-target screening based on LC-HRMS and the environmental risks of the by-products by the QSAR estimations for bioaccumulation, biodegradability and toxicity. Carbamazepine (CBZ), cetirizine (CTZ) and tramadol (TRM), showing the highest exposure levels and detection frequency in Korean rivers, were selected as the test compounds. The gamma irradiation was conducted with a total of 7 steps (0.1, 0.2, 0.3, 0.4, 0.5, 1, 5) kGy of absorbed dose. As a result, all three compounds showed about 50% removal rate at absorbed dose of 1 kGy or less, but 100 % removal rate at absorbed dose of 5 kGy. In total, 23 by-products were identified from parent compounds via suspect/non-target screening. Among them, CBZ-BP271A, CBZ-EP and CBZ-OX were confirmed with reference standard. Other substances were tentatively identified through comparison of MS/MS fragment with library (eg., mzCloud, massBank) or interpretation of MS/MS spectra. By-products were mainly formed through hydroxylation, dehydration, and dealkylation reactions. Based on the identifications the degradation pathway of the by-products was proposed. Through the estimation for the properties, it is expected that most by-products have lower risk in the environment than their parent compound. It indicates that the gamma irradiation can be an alternative tool to eliminate hardly degradable pollutants in conventional WWTP.

3.04.P-Tu079 The Pollution of Yesterday: Historic Landfills As Potential Sources of Aquatic Contaminants Across Scotland

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Until recently, landfilling was the major disposal route for anthropogenic waste in the UK. While emissions from newer landfill sites are controlled, legacy landfills may be an important source of contaminants, including Active Pharmaceutical Ingredients (APIs) and other Emerging Contaminants (ECs), in the environment. However, few monitoring data are available on emissions from these sites and the potential effects on ecosystems. In Scotland, the potential impact of such facilities on the surrounding environment may be most significant in areas where historic landfills are located (e.g., un-lined/capped facilities created before

the Pollution Control Act of 1974). Using Scottish Environmental Protection Agency waste site and capacity data, contaminated land registries and the knowledge of local community groups, a source mapping exercise took place for two regions of Scotland. A total of 81 potential historic and 8 current landfills were identified within 3 km of the coastline or a river in the greater Forth of Forth catchment and within Aberdeenshire and Moray. Water sampling locations (n=46) were selected along rivers potentially affected by the landfill locations as well as other potential sources of APIs/ECs. Duplicate grab water samples were obtained monthly for 1 year across the Forth catchment and for 6 months in Aberdeenshire/Moray (n=912 total samples). Targeted analysis occurred for 70 APIs and ECs using high performance liquid chromatography tandem mass spectrometry generating 63,840 contaminant determinations. Data analysis is ongoing but results to date show that compounds occurring at highest concentrations include the APIs paracetamol (max of 5890ng/L), carbamazepine (2300ng/L), gabapentin (1840ng/L) and metformin (1060ng/L), the lifestyle chemical caffeine (513ng/L) and ECs tris-2-chloroethyl phosphate (389ng/L) and bisphenol-S (107ng/L). Concentrations were highest in the most heavily populated region of the Forth catchment from Dalkeith, through Edinburgh to Falkirk. The most contaminated site was consistently a location on the River Carron (Grangemouth) 7 km downstream from a historic landfill which erodes into the river during high-flow conditions. Here, cumulative concentrations of the studied contaminants (mean= 9380ng/L) were typically 2-3-fold higher than the next most contaminated location. Contaminants with >90% detection frequency included caffeine, carbamazepine, cotinine and gabapentin. This work offers novel insights into historical landfills as API/EC sources.

3.04.P-Tu080 Strategies for Conducting Environmental Fate Studies on UVCB Substances

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The European Chemicals Agency (ECHA) requires chemical substances to be registered, evaluated, authorised and restricted under REACH. The evaluation process may require environmental fate investigations to be undertaken. This may take the form of simulation studies in soil (OECD 307), aquatic sediment (OECD 308) and surface water (OECD 309). In the course of conducting these studies it is a requirement that certain data will be obtained. The required data may include mass balance, the amounts of extracted and non-extracted material, a rate and route of transformation, the extent of mineralisation and identification of transformation products. The use of a radiolabeled substance will facilitate achieving these objectives. Radiolabeling of chemical substances can be challenging. It is relatively straightforward when the chemical substance comprises a single constituent. However, when the chemical is a substance of unknown or variable composition, complex reaction products or biological materials (UVCB) radiolabeling becomes highly challenging. In addition, the conduct of the studies and analysis of samples may also raise many challenges. The poster will explore strategies for overcoming these challenges.

3.04.P-Tu081 Improving Data Quality and Reproducibility in High-Resolution Mass Spectrometry for Identification and Quantification of Micropollutants in Water

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Knowledge about the fate of organic micropollutants in aquatic environments is sparse and the lack of analytical methods is a major research gap. Previous studies mostly focused on the behavior of selected target compounds in individual systems, and the acquired information often cannot be generalized. Given the complex manner in which chemical structure influences micropollutant fate, especially biotransformation, we need to consider a wide range of compounds in order to gain an understanding sufficient for exposure assessment or predictive modeling. In recent years high-resolution (HR) mass spectrometry (MS) techniques such as non-target data acquisition and suspect screening coupled to tandem MS/MS have emerged allowing us to examine thousands of chemicals simultaneously. These techniques are relatively new and important limitations have not been resolved including: i) a lack of reproducibility between HR measurements and instruments ii) a lack of comprehensive spectral libraries hampering high level identification iii) the limited usability of semi-quantitative data produced in screening without appropriate signal correction and reference standards. We optimized an existing method and aimed to advance in all three areas by: i) employing an algorithm-based signal correction method for data processing – supplementing the classical isotope labelled internal standard (IS) method which loses power with increasing numbers of captured compounds and correspondingly increasingly deviant ionization behavior. ii) collecting spectral and retention time (RT) information for over 400 targets in in-house libraries and uploading new data to NORMAN MassBank. iii) confirming the linearity of the corrected instrument response for a large number of known and suspected chemicals. Water samples were analyzed using UHPLC coupled to an Orbitrap HRMS/MS with electrospray ionization. Level 1 and 2a identification was achieved by matching exact mass, spectra and RTs with an in-house mzVault library, standard mixtures and the online databases mzCloud and MassBank. Trials with this new method and river water, municipal wastewater and WWTP effluent regularly delivered >1000 high quality compound hits. Algorithm based signal correction yielded similar or better results than use of ISs in all matrices. The new method substantially improved the linearity of the instrument response and might aid in moving towards full quantification in non-targeted suspect screening analyses.

3.04.P-Tu082 Determination of Multi-Class Emerging Organic Contaminants in Sediments and Soils: Development of a Modified QuEChERS Method Coupled to LC-MS/MS

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CECs and their transformation products are widely distributed in the environment and have been qualified as a risk to human health and environmental ecosystems that urgently need to be addressed. Sorption by soils and sediments plays an important role

in the transport, bioavailability, and ultimately, the fate of these contaminants in the environment. Data concerning the presence and concentration of these chemicals in these matrices have to be evaluated thus, adequate analytical methods for trace level determination have to be developed. The analytical methods for the determination of CECs in soil and sediment are scarce and target only one specific chemical group of contaminants, such as pesticides or pharmaceuticals. Therefore, the development of a multi-residue method that allows the simultaneous extraction and analysis of several classes of contaminants presenting different physicochemical properties is necessary. In this context, we present the optimization of an analytical strategy that combines efficient and reliable extraction, purification, and analysis of a broad range of CECs. Therefore, a new multi-residue and inter-family extraction method for analyzing around 80 emerging pollutants (including pharmaceuticals and personal care products, pesticides, plasticizers, and flame retardants) in sediments and soils based on the QuEChERS method and LC-MS/MS analysis is reported. This procedure involves the extraction of a sample with solvents followed by a liquid-liquid partitioning step. After centrifugation, the extracts are decanted into a tube containing sorbent which constituted a clean-up step called dispersive solid-phase extraction. Different steps were optimized such as the choice of the buffer, the amount of water to add, the choice of the clean-up. The method was fully validated for soil and sediment through the evaluation of recoveries, matrix effects, the limit of quantification, linearity, and precision. Mean recoveries (n=5) were between 70% and 120% with relative standard deviations less than 20% in most of the cases. Using this protocol, a few sediments and agricultural soil samples were extracted and analyzed. The developed method was simple and fast, and good results were obtained in terms of sensitivity, recoveries, and precision. In addition, this strategy could be suitable for non-target screening to provide a more comprehensive overview of the contaminants and their degradation products present in the samples.

3.04.P-Tu083 Analysing ~150 Plant Protection Products in Soil: You Can't Be Everybody's Darling

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Plant protection products (PPP) are applied on agricultural fields to fight crop pests in order to increase crop yields. PPP are designed to be biologically active and to act on target organisms; however, as soon as PPP enter the environment, non-target organisms are exposed as well and may suffer from acute and chronic effects. Whereas knowledge on chemical pollution in surface water bodies located in agriculturally influenced areas is constantly improving, information on soil contamination with agrochemicals is relatively scarce. Therefore, within the *Swiss Action Plan for Risk Minimization and Sustainable Use of PPP* one measure focuses on developing an analytical method to quantify PPP residues in soil. Relevant PPP residues have been selected based on their application rates, environmental behaviour (half-lives, mobility) and toxicity (acute and chronic effects, bioaccumulation potential). The aim of this project was to develop a multi-residue method to be able to quantify ~150 relevant PPP residues in soil with liquid chromatography coupled to mass spectrometry (triple quadrupole) using electrospray ionisation. The main challenge during method validation is the lack of a certified aged reference soil which contains the majority of the target analytes. Only aged soils can reflect extraction efficiencies comparable to those of field soils. Therefore, a “partly-aged” soil was prepared (*RefSoil*) at which the ~150 spiked target analytes had time to interact with the soil matrix for seven days while suspending and mixing. Additionally, five Swiss agricultural soils (*field soils*) were included into the method development which contain native PPP residues. Accelerated solvent extraction and the standardized AOAC QuEChERS method were compared with regard to their extraction efficiencies using the *RefSoil* and the *field soils*. Overall, the extraction comparison led to similar results for the *RefSoil* and the *field soils* in terms of number and identity of the detected substances, the detected concentration ranges and precisions. However, multi-residue methods always rely on a compromise and an appropriate extraction method has to be chosen based on the results of the majority of all target analytes. QuEChERS meets the necessary criteria to be a suitable extraction method for soil monitoring, both from an analytical point of view as well as from a holistic point of view so that soon this method can be applied in routine monitoring to evaluate soil quality.

3.04.P-Tu084 Monitoring of Pesticide Residues in a Potato Field

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Plant protection products (PPP) commonly used for potato crops were monitored in soil of a field under real world conditions. The plot was treated with commercial formulations of 13 fungicides (F), five herbicides (H), one insecticide (I), and one molluscicide. Atrazine (H) was last applied in 2009 and also measured. Of the 21 active ingredients (ai) 19, including eight metabolites (M), were measured. Soil was sampled 14 times between March 2020 and January 2021 with 14 days sampling cycles during the potato growing between April and August. Sampling depths were top- (0-5 cm) and subsoil (5-20 cm). Prior to analysis the soils were dried at 40 °C, crushed and sieved over 2 mm, extracted by accelerated solvent extraction and determined by large-volume injection liquid chromatography coupled to tandem mass spectrometry with electrospray ionization (LVI-LC-ESI-MS/MS). Median concentrations of topsoils ranged from 0.2 ng/g_{soil} (fluzifop-p-butyl, (H)) to 190 ng/g_{soil} (dimethomorph, (F)) and those of subsoils from 0.3 ng/g_{soil} (trifloxystrobin CGA (M)) to 17.0 ng/g_{soil} (dimethomorph). Atrazine, a legacy pesticide, had a median of 1.5 ng/g_{soil} in the top- and 1.6 ng/g_{soil} in the subsoil and atrazine-2-hydroxy, a degradation product of atrazine, 14 ng/g_{soil} at each soil layer. Atrazine and its metabolite were detected in all 14 samples in both soil layers. The same (14/14) holds for prosulfocarb, (H), clomazon (H), metribuzin (H), metribuzin desamino (M), and difenoconazol (F). Hence, of seven analytes found, five were ai and four of them belong to herbicides, of which three were applied four days before the first sampling. Difenoconazol was already detected in April although its application was in May possibly because its residues survived from the previous crop. Observed compound's dissipation rates over time were compared with highest and lowest half-life predictions based on pseudo first order kinetics (DT₅₀) from literature¹. All topsoil concentrations were within concentrations ranges predicted by DT₅₀, only those from fluzinam and fluzifop-p-butyl were lower than predicted. Subsoil concentrations were less in comparison to topsoils because

they depend on the mobility of the compounds in soil, which in turn depends on the soil organic carbon to water partition coefficient. The subsoil concentrations did not fit into the predicted concentration range because residues did not mirror peak concentrations from pesticide applications as the topsoils did. Ref: ¹EFSA (European Food Safety Authority), EFSA Journal 2017; 15(10):4989, 29 pp. <https://doi.org/10.2903/j.efsa.2017.4989>

3.04.P-Tu085 Different Chromatographic Approaches to Determine and Compare Residues of Modern Pesticides in Agricultural Soils in Cuba

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In Cuba, there is little to no information about residues of modern pesticides in agricultural soils despite the fact that pesticides are currently heavily applied. For countries with economic limitations such as Cuba, a proper pesticide management avoiding pesticide residues in the food and environment is all the more important. The main objective of this work is to assess pesticide residues in soils of potato production by gas (GC) and liquid chromatography (LC) coupled with mass spectroscopy and to compare the residues with the pesticide records of 12 different agricultural cooperatives in Mayabeque, Cuba. From 2017-2019 farmers in Mayabeque applied different commercial pesticides in 35 soils and of them, 52 active ingredients (ai) were investigated by LC. Eighteen percent of the ai were detected in the soil by LC (true positive). Around a third of the ai measured by LC were false positives meaning the pesticides were detected but not applied. These are for example clothianidin, dichlorbenzamid (2,6), atrazin-2-hydroxy, carbendazim, linuron, and napropamid. This indicated that residues of pesticides from applications of previous years and for other crops in the same field remained in the soil and/or that the records were not complete. For instance clothianidin is persistent according to the Pesticide Property Data Base¹ and atrazine-2-hydroxy is a degradation product of the persistent atrazine, a triazine herbicide applied in large amounts in the past and now forbidden.² More than half of the ai applied were not detected because they might simply have dissipated by degradation or other processes and therefore fell below the detection limit. Also, this relatively large fraction of no detects is due to the fact that the LC method was tailored for Swiss agriculture and the ai typically applied there. The GC method contained ai that are mostly used in agriculture in the tropics and consisted of 27 ai of which six were detected. In total 16 ai employed were identified and quantified. Eleven of them were detected by both types of chromatographs. This preliminary assessment provides a basic idea about pesticide residues in Cuban soils and contributes to transfer these measurement methods from Switzerland to the lab in Cuba. This allows us to establish for the first time a Cuban measurement technique for the next potato campaign 2020–2022 and to study more ai applied in that period. ¹PPDB, Pesticide Properties DataBase, Version September 2016, accessed Nov 23, 2019, © Copyright University of Hertfordshire <https://sitem.herts.ac.uk/aeru/ppdb/en/> ²Chiaia-Hernandez, A. C.; Keller, A.; Wächter, D.; Steinlin, C.; Camenzuli, L.; Hollender, J.; Krauss, M., Long-Term Persistence of Pesticides and TPs in Archived Agricultural Soil Samples and Comparison with Pesticide Application. *Environ. Sci. Technol.* 2017, 51, (18), 10642-10651.

3.04.P-Tu086 Detection and Occurrence of Diazepam and Clonazepam in Wastewater Samples

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Pharmaceutical pollutants entering the aquatic environment have become a growing environmental concern. These pharmaceuticals are unique pollutants because of their special characteristics and behaviour that cannot be simulated with other organic pollutants. The untreated wastewater effluent that contains pharmaceuticals poses considerable threat to the aquatic ecosystem because of the negative effects of non-target organisms in the water. Recent years have seen a growing concern about the benzodiazepines, as emerging pollutants, and their effects on the aquatic environment. These compounds are nowadays widely detected in sewage wastewater. It is important to increase the emphasis on the characteristics of the benzodiazepines in order to differentiate them from industrial chemical compounds. In this study various solid phase extraction techniques have been employed focusing on isolation of benzodiazepines in wastewater matrices. Employing this methodology has shown improved detection and analysis of diazepam and clonazepam as benzodiazepines. Preliminary results have shown the diazepam concentrations to range between 1 to 9 ppm in seasonal wastewater samples analysed, with higher concentrations during the winter season. In the case of clonazepam, concentrations ranged between 0.4 to 2 ppm. Seasonal characteristics of the benzodiazepines are discussed along the trends observed for the water quality characteristics of the final treated wastewater.

3.04.P-Tu087 Occurrence and Fate of Contaminants of Emerging Concern (CECs) and Their Transformation Products After Uptake by Pak Choi Vegetable

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Nowadays, big quantities of food are lost or wasted, so new ways are being explored to recover and reuse the nutrients, in support to a circular economy. Anaerobic digestion is an ecofriendly solution to recover organic matter and other useful nutritional components from foodstuffs, producing digestate. It can be used in soil and hydroponic agriculture. However, all materials present in the digestate are also preconcentrated. Thus, metals, food pathogens and contaminants of emerging concern (CECs) can reach high concentrations that, afterwards, are recirculated in the food chain when soils are fertilized to grow new crops. These compounds are likely to be uptaken by the crops and, once inside, they can be degraded or transformed, originating transformation products (TPs). Both the uptaken CECs and the formed TPs may pose a risk to the environment and ultimately, to human health. In this study, CECs occurrence in several food waste facilities in Sweden was evaluated and, based on this, twenty of the most

frequently detected compounds were selected (four pharmaceuticals and thirteen per- and polyfluoroalkyl substances (PFAS)). The aim of the work was to evaluate the potential uptake of the selected compounds by Pak Choi vegetable (one of the most consumed in Sweden), but also the identification of their TPs. The crops were cultivated under hydroponic conditions using both mineral and biosolid-based substrates which were irrigated with water spiked with the 20 selected compounds. After harvesting, the samples were extracted with a solid-liquid procedure and bead beating Tyssuelyser. The extracts were analyzed by UHPLC-(QqQ)-MS/MS for the target screening and by UHPLC-Q-Exactive MS for the suspect screening. In the target analysis, different behaviours were observed among compounds (some were less uptaken or transformed), among substrates (biosolid substrate seemed to favour the uptake) and among families (PFAS' uptake was more stable than pharmaceutical's one). In the suspect screening, different transformations processes were confirmed, as hydroxylations, dihydroxylations, methylations or desmethylations, and some phase II transformations as glucuronations, glucosidations, or acetylations. Thus, both target and suspect screenings demonstrated a real uptake and transformation of the adsorbed compounds in Pak Choi.

3.04.P-Tu088 Multi-Residue Pesticide Analysis In Soils of Mayabeque, Cuba: Method Validation and First Applications

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Potato is a prioritized crop in Cuba and irrespective of climate, tropical or temperate, pesticide intensive. Mayabeque is one of the provinces in Cuba with a high intensity of potato plots and was therefore chosen for a pesticide monitoring. Here, the study objectives were (i) to establish an analytical method to determine and quantify modern pesticides, (ii) to evaluate matrix effects (ME) of different soils from tropical but also from temperate climate. The extraction method was QuEChERS and 31 active ingredients (ai) and five metabolites were validated by gas chromatography coupled with a triple quadrupole mass selective detection (GC-TQ). Ten isotope labelled internal standards (IS), spiked prior to extraction, compensated for losses and indicated absolute recoveries. The calibration curves contained seven concentration levels (0, 5, 10, 25, 50, 75, 100 ng/ml) per analyte and were done in acetonitrile with 2.5% formic acid (ACN) or in soil matrix (matrix matched (MM) calibration). Although only red ferralitic soil was sampled, ME was evaluated for four other Cuban soils i.e. skeletal, red ferralitic, grey-brown and yellow ferralitic, and one brown soil from Switzerland. About half of the analytes showed an ME lower than $\pm 20\%$, an average of 35% of the analytes had a medium ME (± 20 to $\pm 50\%$) and only six out of 36 analytes showed a strong ME ($> \pm 50\%$). A skeletal forest soil (SS) without pesticide residues was used for method validation. The Linearity shows linear regression coefficients for all analytes ($R^2 > 0.99$). Absolute recoveries ranged from 44 to 122% (average: 94%, RSD $\approx 26\%$). Limit of detection (LoD) and quantification (LoQ) showed range from 0.0002-1.17 $\mu\text{g}/\text{kg}$ and 0.0008-3.89 $\mu\text{g}/\text{kg}$, respectively. First analyses of soil samples from 12 sites taken before planting potatoes (to) revealed pesticide residues of 22 ai and two metabolites and the sum of concentrations per sample ranged from 4.37 and 78.4 $\mu\text{g}/\text{kg}$. T₀ indicates that pesticides are residues from applications to former crops and are therefore considered persistent. However, the pesticide properties data base (PPDB)¹ indicates some of the ai found (trifloxystrobin, metribuzin) as not persistent in the field. Hence, this work does not only shed light on the influence of different (tropical) soils on the ME but also provides evidence for an updating for tropical soils in the PPDB and serves as a basis to establish a pesticide assessment method in the Cuban lab. ¹Pesticide properties data base (PPDB), <https://sitem.herts.ac.uk/aeru/ppdb/>, accessed 26.11.2021

3.04.P-Tu089 Multitarget and Suspect Analysis for the Identification of Emerging Contaminants in Aqueous Samples: Application to Real Cases

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A combination of multitarget quantitative analysis and suspect screening was developed and applied to influent and effluent samples of two wastewater treatment plants (WWTPs) located in the Basque Country: Galindo and Crispijana, responsible for the wastewater treatment of the metropolitan area of Bilbao (approximately 1 million inhabitants) and Vitoria-Gasteiz (approximately 480.000 inhabitants). The detection of contaminants of emerging concern (CECs) was performed by means of an ultra high liquid chromatograph coupled to an Orbitrap high resolution mass spectrometer (UHPLC-q-Orbitrap). Among the compounds detected antibiotics, antiinflammatories and antihypertensives prevail over pesticides and industrial products, being higher concentrations found in Galindo. In addition, the detection of pharmaceutical compounds used during the pandemic's first months in the COVID-19 treatment including the antibiotic azithromycin, the antimalarial hydroxychloroquine and the antiretrovirals lopinavir and ritonavir may indicate an increase in their use during this period. The complementary suspect analysis enlarged the list of detected compounds with 93 and 16 additional xenobiotic compounds annotated at levels 2a and 3, respectively. Although some compounds showed higher or similar concentrations in secondary and tertiary effluent samples (i.e. azithromycin, clozapine, primidone), in general, a reduction on their concentrations was observed, suggesting some efficiency in their removal. The analytical methodology used in this study demonstrated to be a suitable approach when characterizing the presence of different CECs in WWTP samples, highlighting the need for monitoring studies in aquatic environments as well as the implementation of new advanced technologies in WWTPs for further elimination of CECs.

3.04.P-Tu090 Chemicals of Emerging Concern in Food Waste and Oyster Mushrooms

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In recent years, there has been increasing interest in food production using resource-optimising systems, such as hydroponics and mushroom production, particularly in densely populated areas with low availability of arable soil. The anaerobic digestate from food waste deriving from biogas production plants (BPs) has the potential for use as the plant nutrient source in hydroponic systems as well as for mushroom production. This proposed use of anaerobically digested organic waste as biofertilizer in urban farming creates an urgent need to define occurrence and levels of water-soluble (hydrophilic), (semi-) persistent chemicals of emerging concern (CECs), especially residues from currently used chemicals, drugs, and pesticides. Thus, in-depth knowledge of the chemical of the anaerobic digestate derived from organic waste is a necessity for safe urban food production. CECs pose potential threats to environmental ecosystems and human health. This study examined the characteristics of anaerobic digestate based on food waste in terms of CECs, such as currently used drugs and pesticides ($n=133$). The same substrate was used to test uptake of per-fluoroalkyl substances (PFASs), pharmaceuticals, and parabens by edible oyster mushrooms (*Pleurotus ostreatus*), cultivated on spiked growth substrate. The different biofertilizers from three different BPs had similar CEC profiles. If this profile is found to be spatially and temporally consistent, it can help regulatory agencies to establish priority lists of CECs of highest concern. There are currently limited data available on CECs in food waste and biofertilizers, and more studies are urgently needed. This study provides insights into the uptake of CECs by oyster mushrooms from spiked substrate and potential risks for human consumption. The concentrations of CECs over time depended on the physico-chemical properties of the CECs. It was shown that oyster mushrooms are capable of uptake of some CECs, with higher uptake of PFASs than of parabens or pharmaceuticals. This study showed that oyster mushrooms have the potential to degrade pharmaceuticals and parabens. Thus, more studies are needed on use of oyster mushrooms for remediation purposes through uptake and degradation of CECs.

3.04.P-Tu091 Assessment of Modern Pesticides in Soils of Potato Production in Mayabeque Province, Cuba

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Pesticides application is prioritized for potato cultivation in Cuba. Potato soils receive a mix of 17 - 41 kg_{ai}/(ha*y), with a total of 40 to 45 treatments on average per year. Mayabeque province is a major contributor to potato production¹ and the objective of this study is to assess pesticide residues in potato soils of three different pesticide application levels: intensive, semi-intensive, and agro-ecological (no pesticide application, serves as control) and in three time points: before and after sowing, and at harvest in Mayabeque, Cuba. This is the first study until now that quantifies pesticide residues in Cuban soils taken at 12 sites [intensive ($n=6$), semi-intensive ($n=5$), agro-ecological ($n=1$)] and at three time points. All sites were red ferralitic soils selected following the criteria of potato production (kg/ha) and record pesticides applications. Forty-six pesticides and/or metabolites were analyzed in the soils at the Agrocope laboratory. The extraction was done by Accelerated Solvent Extraction with an organic solvent mix. The identification and quantification of pesticide were made by high performance liquid chromatograph-triple quadrupole mass spectroscopy (HPLC-MS/MS). A minimum of five and a maximum of 24 pesticides were detected in all samples. Clothianidin, atrazin-2-hydroxy, imidacloprid, tebuconazole, S-metolachlor, propiconazole, and azoxystrobin were present in more than half of the samples. These compounds are persistent in soil^{2,3}, which corresponds with the high prevalence. The sum ($\mu\text{g}/\text{kg}$) of pesticide concentrations and the range (min-max) before planting were in intensive systems 174 (63-234), semi-intensive 86 (48-129), agro-ecological 12; at sowing in intensive systems 257 (45-545), semi-intensive 180 (38-477), agro-ecological 11; at harvest in intensive systems 202 (56-429), semi-intensive 253 (159-329), agro-ecological 14. Pesticide concentrations were lowest before sowing and highest after sowing or at harvest. The agro-ecological site had the lowest pesticide concentrations and remained stable at all time points. The concentrations in intensive and semi-intensive managed fields were higher than the control and not quite distinguishable after sowing and at harvest. Pesticide management categories will therefore be revised. This preliminary study serves as a basis to establish a method in Cuba in order to be able, in the long run, to quantify pesticides residues in soil, potato and other crops independently. Refs: [1] ONEI.2015. Agricultura, Ganadería, Silvicultura y Pesca. Edición 2015. <http://www.onei.cu/aec2015.htm>. Accessed 20 May 2015. [2] EFSA, 2019. <http://www.efsa.europa.eu/>. Accessed 15 June 2019. [3] PPDB, Pesticide Properties DataBase, Version September 2016, accessed Nov 23, 2019, © Copyright University of Hertfordshire <https://sitem.herts.ac.uk/aeru/ppdb/en/>

3.04.P-Tu092 Anthropogenic Footprint in Canadian Lake Sediments: The CASE of Trace Organic Contaminants

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Anthropogenic activities introduce continuously large number of trace organic contaminants (TrOCs) into lakes and their tributaries. Sediments are a sink for many TrOCs and their transformation products. Due to the process of sorption / desorption in the water-sediment system, the sediments can expose benthic and aquatic organisms to potentially toxic compounds. The presence of TrOCs in Canadian Great Lakes sediments has been described, but there is no systematic study of their presence in lake sediments across Canada. The goal of the present study was to assess the occurrence (concentrations and detection frequencies) of 47 TrOCs in 230 Canadian lake sediments, and how they vary by province and land cover. Sediments of lakes impacted by different levels of anthropogenic activities were sampled and a methodology based on pressurized-liquid extraction, solid-phase extraction and liquid chromatography-triple quadrupole mass spectrometry was developed to determine the occurrence of 43 TrOCs and 4 transformation products representative of urbanization and agricultural activities. Preliminary results on 28 lakes in the eastern Canada provinces (Ontario, Quebec, New Brunswick, and Nova Scotia) show that at least 1 contaminant was found in each sample, and up to 12 contaminants were found in some lakes. In total, 25 analytes out of 47 were detected in at least 1 lake,

with detection frequencies ranging from 0 to 100% and concentrations from 476 pg/g to 9540 ng/g, respectively. TrOCs concentrations varied greatly from lake to lake, with the highest concentrations found in lakes within watersheds impacted by agricultural activities. The spatial distribution of TrOCs concentrations shows hot spots close to the major cities (Toronto and Montreal) and the heavily agricultural areas of southern Ontario. Significant relationships ($p < 0.05$) between land use and TrOCs concentrations show positive correlations ($r > 0.4$) between atrazine, metolachlor and dimethenamid with agricultural fraction of land use in the watershed, and 5-H-methyl-benzotriazole with urbanization. Our analyses reveal that recreational activities could be a major source of DEET and oxybenzone in more natural lakes. This is the first study to assess the occurrence of TrOC in small (< 100 km²) lakes in Canada.

3.04.P-Tu093 Plants As Bioindicators of Pollution in Riyadh and Abha Urban Areas, Saudi Arabia

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Anthropogenic pollution can assume different forms and impact on all environmental compartments. Tracking pollution through pollutants present in living organisms such as plants, so-called bio-indicators, can be a useful approach to environmental monitoring. The purpose of this study was to investigate and compare plants growing in several industrial or traffic heavily affected areas of Riyadh (large industrial city, >10 million inhabitants, intense traffic) and in Abha (small city, < 760000 inhabitants, near a natural park), as bioindicators and biomarkers of anthropic pollution. Pharmaceuticals and personal care products (PPCPs), perfluoroalkyl substances (PFASs), organophosphorus flame retardants (OFRs) and polycyclic aromatic hydrocarbons (PAHs) were measured in several plant species (e.g. *Digitaria ciliaris*, *Ocimum bacilicum*, *Salsola imbricata*, *Conocarpus erectus*, *Tamarix aphylla*) by liquid-chromatography tandem mass spectrometry (LC-MS/MS) or gas chromatography-mass spectrometry (GC-MS). There were significantly higher levels of contaminants in the plants growing in industrial and traffic heavily areas compared to those growing in more rural environments ($P < 0.05$). Our study shows that pollution has effects on total PPCPs, PFASs, OFRs and PAHs, not only providing evidence of the impact of contamination on ecosystem health, but also pointing the way for the use of plants not only as bio-indicators but also for bio-remediation.

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3.04.P-Tu094 Evaluation of the Relevance of Disinfection By-Products (DBPs) for the Environmental Risk Assessment of Biocides

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Disinfectants are widely used biocidal products which are regulated by the EU Biocides Regulation 528/2012. Their scopes of applications are quite broad and include different fields such as (drinking) water, health care, food processing or construction industry. Among the active ingredients used, highly reactive substances like chlorine, peroxides or ozone are common. During their application, numerous disinfection by-products (DBPs) are formed, especially if organic matter is present. The specific uses and the reaction conditions have great influence on the formation of DBPs. The intended use also determines possible releases of the formed DBPs into the environment. This complex situation is not adequately considered in the guidance within the European Biocides Regulation at the moment. The authorisation of biocidal products is regulated by the EU Biocides Regulation 528/2012 which also requires the assessment of possible DBPs. The existing "Guidance on Disinfection By-Products" is limited to halogen-containing biocidal active substances and selected product types and includes only general scientific strategies for the risk assessment of DBPs. On this basis a harmonized environmental risk assessment (ERA) of biocidal products and their DBPs within the EU is questionable. In order to fill this regulatory gap and develop a feasible regulatory toolkit, the German Environment Agency (Umweltbundesamt, UBA) launched a research project for the consideration of DBPs within the environmental risk assessment of biocidal products (FKZ 3718 65 403 0). In a systematic literature based search potential active substances which are competent to form DBPs have been assigned to already known DBPs also considering the conditions of use in their respective experiments. The obtained results were complemented by an experimental validation. Simulated product applications considering uses in aqueous matrix as well as on hard surfaces under different experimental conditions were analysed for a selection of approx. 60 DBPs. The laboratory simulations were accompanied by analysis of samples from professional applications. The experimental setup and the analytical results of the validation experiments will be presented. Characters text: 2285 (allowed: max. 2500)

3.04.P-Tu096 Proposal of Watch List for Emerging Pollutants Occurring in the Nakdong River, Korea

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Nakdong River, one of the four major rivers in Korea, has been polluted as receiving numerous pollutants from heavily industrialized and populated areas while being used as a drinking water source. Recently, a number of researches have reported occurrences of emerging pollutants (EPs) in the river and requested efficient monitoring and management plans such as EU watch list. The aim of this study is to suggest a watch list through preliminary monitorings of the river and risk assessment based prioritization approach. Initially, 633 candidates were selected as EPs from literature and database searches (e.g., research articles, national chemical database, reports from domestic monitoring program, international regulations and mechanisms for monitoring of EPs). Through the review for applicability of LC-HRMS, 175 substances were subjected to target screening method whereas 458 were qualitatively identified via suspect screening. A risk assessment-based prioritization was applied to substances quantified through target screening, and a scoring system-based prioritization was applied to substances tentatively identified through suspect screening. Sampling campaigns (n=12) were conducted from October 2020 to September 2021, at 8 sampling

sites along the river. As a result targets, 131 substances were quantified above the LOQ. Tris(2-butoxyethyl) phosphate, used as a OPFR, showed the highest mean concentration, followed by alachlor/acetochlor ESA, tris(2-chloroethyl) phosphate and dimethyl phthalate, all with mean concentrations above 100 ng/L. Among the 21 substances whose priority score was assigned through risk-based prioritization, 8 substances (azithromycin, candesartan., clotrimazole, imidacloprid, iprobenfos, irgarol, telmisartan and tris(2-butoxyethyl) phosphate) were identified as high environmental risk. As result of the scoring system for 39 tentatively identified substances, 6 substances (crotamiton, caprolactam, heptaethylene glycol, octaethylene glycol, simetryn and tridemorph) showed a potential environmental risk. In total, 14 EPs was hereby suggested for inclusion in the watch list to extensively monitor and manage the quality of Nakdong river threatened by newly recognized pollutants.

3.04.P-Tu097 Mapping Policy Needs for Scientific Evidence Under the Basel, Rotterdam and Stockholm Conventions *Zhanyun Wang, ETH Zurich, Switzerland*

The Basel, Rotterdam and Stockholm Conventions are key intergovernmental instrument to addressing the global threats of chemical pollution to humanity and the environment. Since their establishment, they has identified many emerging issues such as new Persistent Organic Pollutants, but their national implementation remains challenging, particularly in low- and middle-income countries. Concerted action is needed to assist countries that are Parties to the Conventions in implementing their obligations, including enhancing scientific support. This analysis reviews various policy needs for scientific evidence under the Conventions that warrant attention and research from the broad scientific community, including disciplines of both natural and social sciences, as well as research funding agencies. For example, field studies measuring legacy and emerging contaminants under the Conventions are much needed. It further provides practical guidance to scientists and practitioners to ensure the visibility and accessibility of their work for the Conventions' implementation, in addition to conducting timely research.

3.04.P-Tu098 Multi-Stressor Approach on the Impact of Human Activities in Estuarine Systems - Occurrence of Emerging Contaminants in Fish Aquacultures and Concerns Regarding Human Consumption

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The need to understand pollutant mixtures' impacts on organisms is an ongoing discussion, being mixture experimental exposures with environmentally relevant concentrations encouraged. An approach to naturally occurring exposure conditions will allow to detect interactions between contaminants that can be overlooked when performing single-contaminant experiments. Coastal environments are truly rich environments, sustaining a multitude of intricated trophic relations, while providing numerous services for humankind. However, these systems are usually highly impacted by human activities. One common practise in coastal systems in Southern European countries is the use of land for aquaculture and, in some countries, semi-intensive fish rearing is common. If, on one side, this technique may provide a natural factor in fish production, potentially increasing its market value, it also means that the organisms are subjected to the cocktail of contaminants released into the waters by human activities. It is, then, urgent to understand how these contaminants may be, together, affecting fish, and, eventually, human health. Hence, a multi-stressor screening on three matrixes (fish, water and sediment) at semi-intensive fish aquaculture facilities in two Portuguese estuaries was performed, to provide a database of emerging contaminants (EC) found in the rearing systems, that may be used in further works to determine the metabolic impacts of EC in organisms and, eventually, in human-beings as consumers. Three main EC groups were defined for the screening – microplastics (MPs), polycyclic aromatic hydrocarbons (PAH) and pharmaceutical and personal care products (PPCP). The identification of MP polymers is being carried out through ATR-FTIR. The identification of PAH and PPCP is achieved through GC-MS. Identification and quantification of EC in all samples is ongoing. First results show that i) polyethylene (with different densities) and nylon are the most frequent MPs in all matrixes, found in higher amounts in fish gut and sediment; ii) PAH are more frequent in sediment; iii) a wide variety of PPCP can be found, such as additives, anti-depressive drugs and sunscreen, being apparently abundant in all matrixes. The fact that all EC are found in fish aimed for consumption contributes with further and alarming knowledge for legislative and regulatory processes that effectively act towards lowering the impact of human activities, safeguarding ecosystems and human health.

3.04.P-Tu099 Effect-Based Characterization of Polycyclic Aromatic Compounds (PACs) During In-Situ Thermal Remediation of a Former Gasworks Site

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In-situ remediation, in comparison to ex-situ remediation, is getting more and more attention as both research and technology advances as well as some advantages in-situ remediation poses over ex-situ remediation, e.g., limiting human exposure, lower cost etc. Soil remediation plays an integral role in achieving a healthy ecosystem. A group of compounds that are often present in high concentrations at industrial sites are polycyclic aromatic compounds (PACs). While polycyclic aromatic hydrocarbons (PAHs), especially the 16 US-EPA priority PAHs, have been studied pre- and post-remediation, little is known about the potential for thermal treatment to remove other PACs. In this study we are going to investigate changes in the composition of PACs, including PAHs, oxy-PAHs, alkylated PAHs, and heterocyclic compounds (NSO-PACs) and bioactivities in soil at a historically contaminated gasworks site in Sweden after in-situ thermal remediation. We will use a three-pronged approach, 1) targeted chemical analysis, 2) a battery of in-vitro reporter gene assays, and 3) effect-directed analysis (EDA). With the EDA approach, we aim to i) study the composition of the contaminants present in the samples (pre- and post-remediation), ii) determine any changes

in contamination profile, pre- and post-remediation, and iii) identify bioactive PACs, both novel and known. The results from this study will give a better understanding on degradation of PACs and formation of potentially toxic metabolites of PACs in soils during thermal remediation. Therefore, gaining more data and knowledge to further develop risk assessment models for PAC-contaminated soils.

3.04.P-Tu100 Calibrating a Passive Air Sampler for Semi-Volatile Organic Compounds Using Continuous Active Air Sampling and Long Deployment Periods

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The use of passive air samplers (PAS) for atmospheric semi-volatile organic compounds (SVOCs) has expanded over the past decades. Confident use of a PAS requires knowledge of its uptake kinetics in their dependence on meteorological conditions and chemical properties and of the limits of linear uptake of more volatile SVOCs. Such knowledge is gained through calibration studies involving the side-by-side deployment of active and passive air sampling techniques. Here we describe a calibration of the XAD-PAS, which uses styrene-divinyl-benzene-copolymeric resin as a sorbent. The resin's high capacity increases the likelihood of linear uptake, including for relatively volatile SVOCs and during long deployments. 24 XAD-PASs were deployed on the campus of the University of Toronto Scarborough in the Eastern suburbs of Toronto in June 2020 and duplicates retrieved at monthly intervals. From June 2020 to May 2021, 48 consecutive week-long active samples were taken with a mid-volume pump. The gas and particle phases were collected on PUF-XAD resin sandwiches and glass fibre filters, respectively. Numerous field blanks were collected. One duplicate of each pair of PASs, all blank samples and the PUF-XAD resin sandwiches have been extracted, concentrated, and are being analyzed for polycyclic aromatic hydrocarbons, halogenated flame retardants, polychlorinated biphenyls, organochlorine pesticides, organophosphate esters, and neutral per- and polyfluoroalkyl substances. Due to relatively low partitioning ratios between XAD resin and the gas phase, more volatile compounds may have reached equilibrium during the length of the calibration experiment. For example, the amount of 2-methylnaphthalene sorbed to the XAD-resin reached a maximum of ~5 mg after half a year of deployment and remained relatively constant during longer deployments. Whereas, the sorbed amount of less volatile PAHs, such as fluorene, increased with the deployment length throughout the entire year. Sampling rates can be derived using the slopes of the linear regressions through the curves of effective sampling volumes over the deployment period. For example, the sampling rates of ?-TBECH, ?-TBECH and BDE-47 are 2.0, 1.7 and 0.8 m³ day⁻¹, respectively. It is hoped that this "gold standard" calibration experiment will succeed in greatly expanding the knowledge base on sampling rates and lengths of the linear uptake period for a great variety of legacy and emerging SVOCs in the XAD-PAS.

3.04.P-Tu101 The Role of Passive Sampling in Environmental Monitoring - Theory & Recent Advancements

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Passive sampling is an established technique in the monitoring and characterisation of Volatile Organic Compounds (VOCs). Employed across various fields such as personal exposure monitoring, large-scale environmental studies and indoor air monitoring. Passive sampling provides a cheap, convenient and reliable method of characterising a range of VOCs across several samples in large-scale air monitoring programmes. Passive sampling possesses several favourable qualities to alternative methods, such as no pump requirements and a non-invasive design. Further to this, early sorbent-based passive samplers were required to undergo solvent extraction as a preparation step before analysis. The solvent extraction limited air-speed effects at the surface of the badge, preventing the stable conditions required for passive sampling. Recent developments allow analytes to desorb from the sampler via thermal desorption (TD), improving sensitivity and accuracy that were unachievable through earlier sorbent-based sampling devices. Within this presentation I will detail the latest TD-compatible passive samplers, exploring axial and radial sampling technologies and how these technologies are advancing environmental monitoring.

3.04.P-Tu102 Development of Active-Passive Sampling (APS) Strategies for Dynamic Speciation Analysis and Ecotoxicity Evaluation of Psychoactive Substances and Their Metabolites in Wastewater

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Wastewater analysis of drugs and their metabolites can provide information on drug use and abuse in general populations. Conventionally, 24 h composite samples are analyzed and the chemical reactivity of the compounds within the wastewater matrix is ignored. In practice, these compounds are likely to associate with the diverse particles present in the wastewater matrix. Negligence of such interactions will lead to erroneous estimations of analyte concentrations. To address this issue, this project employs innovative active-passive sampling (APS) strategies to quantify psychoactive substances in wastewater, taking into account their chemo-dynamic behaviour. The interpretation framework will be supported by determination of analyte-particle interactions with a range of particles. The APS device incorporates a controlled hydrodynamic flow of the sample matrix across selective sorbents which accumulate the target compounds. APS can be used in equilibrium mode to estimate the equilibrium analyte concentrations, e.g., the freely dissolved form; and in non-equilibrium mode to provide information on the kinetic features of their chemical forms in wastewater matrices. The performance of the APS will be compared with conventional 24 h composite sampling in both influent and effluent flows of wastewater treatment plants. A novel passive dosing strategy will also be explored for the ecotoxicological risk assessment of the drugs and their metabolites via a battery of toxicological tests.

3.04.P-Tu103 Beyond 1,2,4-Triazole - Extending the Field Scale Leaching Assessment of Triazole Fungicides by Suspect-/Non-Target-Screening

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Triazole compounds are commonly used fungicides used both for combating fungal diseases in crops and as seed dressing. Recently, there has been increasing concern that 1,2,4-triazole, a major degradation product of several triazole fungicides, may leach to groundwater in concentrations exceeding the 0.1 µg/L threshold limit of the European Union. 1,2,4-triazole is detected in 21% of Danish monitoring wells with exceedance of the 0.1 µg/L threshold in 3.4% of the wells. However, this is the only transformation product hitherto included in targeted monitoring of the triazoles although several transformation products are mentioned in the respective triazole EFSA conclusions. The aim of this study was therefore two-fold: i) to monitor closely the leaching of triazoles and the transformation product 1,2,4-triazole to drainage and groundwater following multiple triazole applications on different crops (target-analysis by LC-MS/MS), and ii) to study the presence of both suspected and hitherto unknown transformation products from the triazole fungicides in drainage and groundwater by HRMS suspect- and non-target analysis. The study was performed on drainage and groundwater samples collected from five fields included in the Danish Pesticide Risk Assessment Programme (PLAP). Since 1999, the PLAP programme has been monitoring leaching of pesticides and transformation products to drainage and groundwater. Additional information about monitoring design and sampling methods is available online (www.pesticidvarsling.dk). The five test fields included in the PLAP programme are cultivated with crop rotations in accordance with conventional agricultural practice in the area and triazole fungicides have been applied consecutive times at the maximum permitted dose.

3.04.P-Tu104 Anthropogenic Gadolinium in Surfacewaters, Determination of Mri-Contrasts Using Ic-Icp-Sf-Ms

Marcel Kotte, RWS, Netherlands

Gadolinium-based contrast agents used in magnetic resonance imaging are difficult to impossible to remove in wastewater treatment plants, and may enter groundwater production wells and hence municipal tap water via bank filtration. As Rijkswaterstaat is responsible for surface-waters used in the production of drinking water, it has interest in knowledge about substances in its water-management area. Over a period of time, starting from January 2021, RWS has gathered information on the presence of Gd-based contrast agents. Together with information on the concentrations of other rare earth elements in surface water, RWS can provide an insight in the anthropogenic Gd. The poster will go into the analytical method used to determine Gd-based agents. Ion chromatography coupled with inductively coupled plasma, sector field mass spectrometry is used. Results will show that mostly macrocyclic Gd-based agents are present. This is in line with expectations, because these substances are used in the Netherlands. One linear compound is present which is also in line with expectations as this compounds is still used in Germany.

3.04.P-Tu105 Air-Sea Exchange and Atmospheric Deposition of Phthalate Esters in the South China Sea

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Phthalate esters (PAEs) are synthesized organic chemicals and have been used on a large scale as plasticizers and additives for more than 80 years. Upon their excellent characteristics, PAEs are extensively applied in manufacturing of resin and polymer products, building materials, personal care products. As PAEs are physically added in the products, they can leach out during the production and application, and especially from disposition of plastic waste. The continuous emissions of PAEs have led to their ubiquitous distribution and abundance in the global environment. In this study, we have determined nine PAEs in air and seawater samples from the South China Sea. Stationary air sampling has been performed at the Yongxing Island Marine Research Station of the South China Sea Institute of Oceanology from 17 May 2018 to 21 July 2019. Ship-bound sampling was carried out on board German research vessel Sonne along the transect from Singapore to Hong Kong from 2 August to 2 September 2019. All air and seawater samples were spiked with internal standards and extracted using a modified Soxhlet system in a clean lab. The sample analysis was performed with Agilent 8890A gas chromatography coupled to Agilent 7010B tandem mass spectrometry (GC-MS/MS). The concentrations of PEs are from below the method detection to 13 ng/m³ in air samples, and from 1.1 to 23ng/L in seawater, respectively. DiBP, DnBP and DEHP are the predominate PAEs in both air and water samples. Concentrations of PEs in air samples from Yongxing Island were supposed to be impacted by the monsoon, which leads to significant seasonal variations. Air-sea exchange and atmospheric particle deposition fluxes are calculated with paired gas/water concentrations and particle-bound fractions. The results showed air-to water deposition is the major process, which make the Southern China Sea being an important sink for PAEs.

3.04.P-Tu106 Accumulation and Chronic Effects of Cobalt on the Gastropod *Radix balthica*

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Gastropods are widespread mollusk living at the bottom and in shallow water of freshwater systems. First consumers of periphyton and plants, they represent an important source of food for large crustacean and fish. Toxic effects due to contamination such as metal increase might thus impede food sustainability of ecosystems. Gastropod shell has the interesting feature to grow

throughout their life by integrating some of the chemical elements present in their environment, including metals. Moreover, direct correlations have been observed between metal concentrations in the soft bodies of mollusks and those present in their ambient environment. The present study aims thus to examine the relationships between metal concentrations in the environment, in the gastropod soft tissue and in their shell. Here, cobalt (Co) is the metal of interest due to its increasing use in recent technologies of transport or energy production. Co effects towards gastropod growth and reproduction are also examined. To that end, the gastropod *Radix balthica* was exposed to increasing Co concentrations (6, 30 and 60 $\mu\text{g}\cdot\text{L}^{-1}$ Co) during 28 days using outdoor mesocosms (PERL, TotalEnergies) filled with the Gave de Pau water. Water and organisms were collected after 7, 14, 21, 28 days of exposure. The physicochemical parameters (pH, conductivity, oxygen and turbidity) were also monitored. Water was analysed for anion, cation and dissolved organic matter concentrations. Gastropod shell measurements (length and width) were performed at each sampling step whereas the number of egg masses was additionally monitored. Total metal concentrations were analyzed in the whole shells and soft bodies using acid digestions. Finally, microchemical analyses of metals in the shells are currently performed using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) and Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS). At day 28, the mortality (13, 19, 22 and 27 %) and the number of egg masses (21, 30, 44 and 56) increased with the Co concentrations (control, 6, 30 and 60 $\mu\text{g}\cdot\text{L}^{-1}$ Co, respectively). The number of neonates was higher in the control (193) than in Co enriched mesocosm (113, 10 and 58 for 6, 30 and 60 $\mu\text{g}\cdot\text{L}^{-1}$ Co, respectively). Finally, Co was measured by LA-ICP-MS at a concentration of $1.24 \pm 0.27 \mu\text{g}\cdot\text{g}^{-1}$ in the gastropod shell exposed to 60 $\mu\text{g}\cdot\text{L}^{-1}$ Co at day 28. Those results will allow us to determine if gastropod shell can be a good bioindicator of metal in freshwater.

3.05 Advancing analytical and experimental approaches for organic chemicals (Part I)

3.05.T-01 Characterising the Organohalogen Iceberg: Comprehensive, Multi-Halogen Mass Balance Determination in Sludge

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Organohalogen compounds (OHCs) comprise a wide range of fluorine-, chlorine-, and/or bromine-containing substances which are mostly anthropogenic and often have persistent, bioaccumulative and toxic properties. Currently, more than 30 000 individual OHCs, containing one or more halogens, are registered with a unique CAS number. Capturing this large number and diversity of chemicals in a single targeted method is challenging, leading to concerns that exposure to OHCs is underestimated. For this reason, there is growing interest in so-called “organohalogen mass balance” experiments, which seek to quantify the fraction of unidentified organohalogen in samples through a combination of mass spectrometry-based targeted analyses (both gas and liquid chromatography) and combustion ion chromatography (CIC)-based extractable organohalogen measurements. In this study we developed a multi-halogen CIC method for simultaneous determination of extractable organofluorine, -chlorine and -bromine in wastewater treatment plant (WWTP) sludge. The method was applied together with targeted analyses on samples from a WWTP in Stockholm. Targeted analyses included 16 per- and polyfluoroalkyl substances (PFAS), chlorinated paraffins (CPs), and 5 chlorinated and 38 brominated flame retardants (Cl-FRs and BFRs). Known extractable organohalogen (EOX) concentrations were calculated by summing the halogen equivalent concentrations from all OHCs measured by targeted analysis and then comparing these values to CIC-based EOX concentrations. 16 PFAS contributed only ~2% of the EOF concentration, with the greatest contribution attributed to perfluorooctane sulfonate (PFOS; $7.0 \pm 0.6 \text{ ng/g dw}$). Likewise, 64% of organobromine levels were accounted for by 38 BFRs, with BDE209 ($130 \pm 16 \text{ ng/g dw}$) and bis(2-ethylhexyl)tetrabromophthalate (BEH-TEBP; $83 \pm 6 \text{ ng/g dw}$) representing the most dominant substances. To the best of our knowledge, this study represents the first multi-halogen mass balance experiment conducted on sewage sludge samples. These data provide a comprehensive picture of both the known and unknown portions of the OHC “iceberg” in sludge and show that organohalogen concentrations are severely underestimated, especially for organofluorine and -chlorine compounds. Future work will apply high resolution mass spectrometry-based non-target screening to elucidate this unknown fraction.

3.05.T-02 Unravelling Chlorinated Paraffins; Characterising an UVCB and Evaluating Its Hazard Potential

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UVCBs are compounds of unknown or variable composition, including complex reaction products and biological materials. The complex composition makes the characterisation and assessment of their (bio)degradation, bioaccumulative and toxic potential very challenging. The hydrophobic nature of most constituents further complicates the hazard and risk assessment. This study presents a novel approach for the isolation, collection, enrichment and characterisation of UVCB constituents. As proof of principle chlorinated paraffins (CPs) are used. Our approach applies GC fractionation in combination with various analytical methods, including nuclear magnetic resonance spectroscopy and GC×GC. With this approach it is possible to analyse a whole GC chromatogram by NMR and it lays the foundation to develop methods for obtaining purified single UVCB constituents. We also explored the possibility of generating biodegradation kinetics data for CPs and linking the fate data to toxicity data, a so called fate-directed toxicity approach. For generating biodegradation kinetics data a promising existing partitioning-based method has been applied that is able to determine biodegradation kinetics of hydrophobic organic hydrocarbons at environmentally relevant low concentrations. For generating toxicity data the Zebrafish (*Danio rerio*) early life test was applied to assess developmental toxicity and neurotoxicity.

3.05.T-03 Pyrolysis-Gc-Hrms an Application for the Identification and Quantification of Organic Plastic Additives

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Environmental pollution from plastics and microplastics (MPs) is a threat to the environment and wildlife on multiple aspects regarding exposition of different hazards. One of them is due to the chemical impact of the organic plastic additives (OPAs). Various additives can be added to polymers during the manufacturing process to modify and improve their physical properties. The analysis of OPAs has shown a growing importance since their use has become controversial as some of the additives were found to be toxic to marine organism and humans. In order to have a better understanding of the chemical impact of those additives, it is important to identify and, ideally quantify the additives included in the polymeric matrices. The analysis of OPAs and polymers is an analytical challenge due to the diversity of their chemical composition, the fact that they are most of the time mixed, and the numerous steps needed for the sample preparation with "conventional" approaches such as solvent extraction (SE). Thus, implementing easy and quick screening analysis methods, able to analyse plastic additives, have become a focus of interest. Pyrolysis coupled to a gas chromatography and a high-resolution mass spectrometer (Py-GC-HRMS) is an analytical tool that enable a relatively quick and easy analysis. This technique minimize or even remove the steps for sample preparation, limiting their potential contamination prior the analysis. The goal of this study is to develop a method using Py-GC-HRMS to identify and, if possible, quantify OPAs included in a polymeric matrix. A single method for the analysis of various selected plastic additives has been implemented and benefits the constitution of a database in GC/MS-Orbitrap with a wide variety of plastic additives. This method has shown its ability to characterise several additives in plastic packaging samples. The development of the quantitative method is still on going.

3.05.T-04 Why DO Some "Blank" Samples Show Toxicity in Effect Directed Analysis?

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Testing toxicity on environmental aquatic samples and using different approaches of effect directed analysis (EDA) to identify the substances causing toxicity, is an important tool to identify new and emerging toxicants of environmental concern. To measure toxicity, however, samples have to be up-concentrated to obtain an extract of the organic pollutants that can then be diluted into the media designed for the biological toxicity test. For the upconcentration of the environmental sample, different materials for solid phase extraction (SPE), and different solvent compositions are typically used for optimal elution of the sample. Paramount for the quality control of the EDA is the ability to run non-polluted water samples through this sample preparation procedure without detecting any toxicity in the biological assays at the relative enrichment factors (REF) used. In an ongoing project exploring the potential of virtual EDA (Vandalf-project), we have observed toxicity in both algae and daphnid bioassays from blank samples dosed at REF values up to 20-30. These results rose the question of what was causing the toxicity in our "blank" samples? We discovered that both ammonium added in the eluent mixture, and metals occurring in blanks could induce toxicity to algae and daphnids, respectively. We will present data on the frequency of blank sample toxicity, sources of the toxicity, and how it can be avoided and or dealt with when doing effect directed analysis.

3.05.T-05 TIMFIE Sampler - a New time-integrating, Active, Low-Cost Sampling Device for Quantitative Determination of Organic Micropollutants in Whole WATER

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Sampling techniques that enable quantitative determination of whole water concentrations are vital to assess the status of different waterbodies with respect to organic micropollutants (OMPs). Since OMP concentrations in aquatic environments are episodic, time integrated sampling is needed to estimate average concentrations and chronic exposure of biota. Time integrated sampling with automatic, electric samplers can be prohibitively expensive thus limiting the number of locations sampled and decreasing operational flexibility. Time integrated measurements based on passive sampling do not measure total concentrations and are not convincing for quantitative analysis. As a result, grab sampling is still the dominant sampling technique, despite its inadequacy for estimating average concentrations. To address these shortcomings, the Time Integrating, Micro Flow, In-field Extraction (TIMFIE) sampler was developed. This quantitative sampler is constructed from inexpensive laboratory consumables and requires no battery or power supply. A 120-mL single use syringe, connected to a flow restrictor, is set under negative pressure by pulling and locking the piston. This enables a low ($\mu\text{L}/\text{min}$) flow through SPE cartridges, where target compounds are continuously extracted over one or two weeks. The extracted water ends up in the syringe where sample volume can be determined, thus enabling quantitative analysis. The large number of available SPE adsorbents enable simultaneous extraction of compounds having very different physicochemical properties. TIMFIE was originally developed for pesticides but has been used to measure other OMP classes in whole waters, i.e., PFAS, pharmaceuticals, industry chemicals and personal care products, using four different LC-MS/MS multimethods. Furthermore, the water collected in the syringe opens up new possibilities to determine average free concentrations of other substance classes, e.g., metals, and possibly nutrients. Thanks to its small format and robust, low-tech construction the TIMFIE sampler can be mounted in many different ways, to support various needs, including winter sampling. Experiences from method development and validation, as well as a field study performed in five different regions in Sweden during 2021 will be presented.

3.05 Advancing analytical and experimental approaches for organic chemicals (Part II)

3.05.T-06 In Situ Vertical Profiles to Measure Seasonal Variation in Dissolved HOC Concentrations at the Sediment-Water and Air-Water Interphases

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Hydrophobic organic contaminants (HOCs) are ubiquitously present in the aquatic environment, and cycle in the environment between different environmental phases. In this study low density polyethylene (LDPE) passive samplers were used to attain *in situ* vertical profiles from sediment-water and air-water interphases, to study the seasonal variation in air and water concentrations of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), and to assess their diffusive mass transfer at the air-water and sediment-water interphases. Seasonal variation in surface water and air concentrations was observed, with higher concentrations of PAHs in the autumn. The observed difference is likely induced by higher combustion-derived PAHs sources during autumn. The opposite was observed for PCBs, which implemented higher concentrations in surface water and air during the summer period. Elevated PCB concentrations in air during summer may be due to mass transport from urbanized areas or from re-volatilization from water. Upward diffusive fluxes from water to air for PCBs (excluding CB-28 and CB-110) were observed in this study, potentially contributing to the elevated air concentrations at the sampling site. Desorption fluxes from air to water was observed for PAHs at both seasons. Enrichment of the HOC concentrations near the water surface was observed, indicating that the air-water profilers can provide high resolution vertical measurement of HOCs in the subsurface waters, sea-surface microlayer and in the air above. Thermodynamic disequilibrium was observed between sediment pore-water and bottom water, with upward release fluxes from the sediment. These results indicate that when the primary emissions of HOCs have decreased, sediments at the coastal areas can start acting as a secondary sources of HOCs to the water phase. Passive sampling at the sediment-water and air-water interphases proved as an effective method to assess the concentrations and fluxes of HOCs in the coastal Baltic Sea and to detect seasonal variation in concentrations. *In situ* passive sampling campaign using the same passive sampling polymer in multiple environmental phases provides advantages for assessing the environmental fate, for example by allowing direct comparison of the equilibrium polymer concentrations to assess the direction of the air-water and sediment-water exchange.

3.05.T-07 Probing the Thermodynamics of Biomagnification in Captive Polar Bears by Equilibrium Sampling of Dietary and Fecal Samples

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Biomagnification is the process through which organisms can have higher lipid-normalized concentrations of hydrophobic contaminants in their bodies than are prevalent in the food they eat. The underlying mechanism is a decrease in volume (V) and fugacity capacity (Z) of the food for the biomagnifying contaminants during digestion and assimilation in the gastrointestinal tract. In an earlier proof-of-concept study, we used equilibrium sampling with silicone films to noninvasively derive the thermodynamic limit to a canine's biomagnification capability (BMF_{lim}) by determining the ratio of the $V \cdot Z$ products of undigested and digested food. Low contaminant levels prevented the determination of the fugacity (f) or chemical activity of contaminants in food and feces. For polar bears from the Toronto Zoo, fed on fish and seal oil, we were not only able to measure the Z -value of diet and feces by equilibrium sampling, but also the increase in the f of native polychlorinated biphenyls (PCBs) upon digestion, providing incontestable proof of the process of gastrointestinal biomagnification. We could also confirm that equilibrium sampling yielded similar Z -values for PCBs originally present in food and feces and for isotopically labelled PCBs spiked onto those samples, so that the determination of f even in samples with small size and low contaminant levels becomes possible as long as the concentration of the compound in the sample (C_{native}) can be quantified (i.e. $f = C_{native}/Z_{spiked}$). For zoo bears eating a diet rich in lipids (24 %, as fed basis), an observed BMF_{lim} of ~ 171 indicates a very high biomagnification capability, which can be attributed to reductions in both Z (Z_D/Z_F from 53 to 113) and V ($V_D/V_F = 2.4$). Our study highlights the importance of a high fat diet and a high lipid assimilation efficiency (99 %) for achieving high BMF_{lim} . A PCBs uptake efficiency of 98 % indicates that contaminant concentrations in the zoo bears are still far from steady state, thus resulting in a biomagnification factor ($BMF = f_{feces}/f_{food} = 3$) that is much smaller than the BMF_{lim} .

3.05.T-08 In-Tube Passive Dosing for Flow-Through and Large-Volume Experiments

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Passive dosing is increasingly used to control exposure concentrations of hydrophobic organic chemicals (HOCs) in small sized and short term aquatic tests. However, there are increasing demands for conducting aquatic toxicity and bioconcentration experiments in larger test systems and for longer test durations. The challenge of upscaling passive dosing to larger test systems is that mass transfer from the passive dosing donor to the exposure medium is diffusion limited. Longer test durations can lead to the formation of biofilm that can limit mass transfer or even degrade the test substance. This study introduces in tube passive dosing (in tube-PD) as an approach to control HOC exposure in larger test volumes and flow-through experiments: Aqueous medium is dosed by pushing it through a tube holding HOC loaded silicone rods. We equipped a 6 meter PTFE tube (ID 10mm) with four parallel silicone rods (OD 3mm) to accommodate water flows from 0.2-120 L/h, and developed a mass transfer kinetic model to capture the chemo-dynamics in the system. The first experiment was conducted with fluoranthene, where aqueous concentrations were measured by molecular fluorescence at different (1) flow rates, (2) water volumes and (3) positions within the tube. The second experiment was conducted with a complex petroleum mixture (cracked gas oil, UVCB), where concentrations of 22 mixture constituents were measured by solid phase microextraction coupled to GC-MS. The results showed that the system

provided very stable and reproducible concentrations over extended periods of time, which were at equilibrium for flows < 10 mL/min and steady state at higher flows. The results were consistent with the mass transfer kinetic model. A long-term test showed that approximately 1.5 m³ water can be dosed before fluoranthene concentrations declined by 20%. The developed in tube-PD can easily be combined with various types of toxicity, bioconcentration and other experiments. The physical separation of dosing system and test vessel can here minimize biofilm formation on the PD donor. A mass transfer kinetic model was developed, which not only was used to fit the experimental data but also provided mechanistic insight of the partitioning kinetics. This model can guide the future design and optimization of in tube-PD systems to meet the various demands in aquatic testing and research.

3.05.T-09 Expanding the Applicability Range of the Generator Column Technique for Measuring the Octanol-Air Partition Ratio to Volatile Substances

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Bioaccumulation modelling has identified a lower limit of ~5 for the log of the octanol-air partition ratio ($\log K_{OA}$) of chemicals that potentially bioaccumulate in air-breathing organisms. While hundreds of measured $\log K_{OA}$ values exist, few are close to that threshold, because existing techniques are not well suited to this volatility range. Here, we modified the generator column method to allow for K_{OA} determinations of more volatile compounds. In a generator column experiment, octanol saturated air flows through a column containing an octanol solution of the analytes of interest. Chemicals evaporate into the air stream until equilibrium is reached. They are collected from air and quantified. The K_{OA} is calculated from the concentration in octanol (C_O) and the volume of air passing through the column (V_A). For less volatile compounds a large V_A is equilibrated with the octanol so that the amount of chemical in air can be quantified. This necessitates high flow rates of 50-100 mL/min and long experimental run times. For volatile chemicals, a smaller V_A needs to be equilibrated with octanol to prevent chemical depletion from the column. We reduced flow rates to 5-15 mL/min and experimental run times to 30-60 minutes. The concentration of less volatile chemicals in the octanol in the generator column is assumed to remain constant during multiple experiments lasting many hours; it is often not even measured but inferred. Because volatile chemicals are more likely to be lost from the octanol, we extract and measure the amount of chemical in the column at the end of an experiment and calculate the C_O during each run by interpolating between the initial and final C_O . By recording K_{OA} values for hexachlorobenzene that agree with earlier measurements we confirmed our ability to obtain reliable data with the generator column technique. $\log K_{OA}$ values for tri-isopropyl phosphate, tetradecene, 1,3-dichlorobenzene, 1,2,3,4-tetramethylbenzene, *trans*-decalin, 2,6-dimethyldecane, and hexylcyclohexane have been obtained with the modified technique. Good agreement with predictions made with poly-parameter linear free energy relationships lends confidence to these data. Reducing gas flow rates and experimental run times and quantifying the concentration in the octanol phase are key to obtaining reliable K_{OA} data when applying the generator column technique to volatile compounds.

3.05.T-10 Understanding the Effects of Temperature on Pesticide Plant-Air Partitioning and Foliar Penetration

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Plant-air partition coefficient ($K_{\text{plant-air}}$) values are needed to understand and predict pesticide volatilization and persistence in agroecosystems. While temperature is expected to increase chemical volatilization from surfaces, there are several competing processes on the leaf surface and each is affected by temperature in different ways. Of particular interest, increasing temperatures can lead to increased sorption to the leaf surface but can also increase diffusion into inner leaf layers. Since both of these processes could result in less chemical available for volatilization, the effects of temperature on volatilization may not be as easy to predict as expected. The objectives of this work were to measure $K_{\text{plant-air}}$ values and foliar penetration for two current-use insecticides on alfalfa (lucerne) leaves at a range of temperatures and to use this data to better understand how sorption and foliar penetration effect plant-air partitioning. The insecticides investigated were chlorpyrifos (an organophosphate, studied as both the active ingredient alone and in a formulation) and γ -cyhalothrin (a pyrethroid, studied as the active ingredient alone). $K_{\text{plant-air}}$ values were measured using a solid-phase fugacity meter. Following the 16-h experiments, a portion of the leaves were used for foliar penetration experiments. This method involved successive extractions in which ethanol-extractable (surface associated), hexane-extractable (cuticle associated), and remaining (tightly bound) fractions of chemical were collected. We found that temperature had an opposing effect on the $K_{\text{plant-air}}$ and foliar penetration trends for the two pesticides. In sum, chlorpyrifos behaved as expected, with both increasing volatilization and diffusion to inner leaf layers with increasing temperature. However, γ -cyhalothrin displayed decreasing volatilization and increasing sorption to the leaf surface with increasing temperature. Nonetheless, we found that $K_{\text{plant-air}}$ values increased as the amount of chemical in the ethanol-extractable layer increased for both chemicals. Thus, we concluded that increasing temperature leads to changes in leaf surface properties that have complex effects on the interplay of volatilization, sorption and diffusion, resulting in different possible trends in $K_{\text{plant-air}}$ with temperature for different chemicals.

3.05 Advancing analytical and experimental approaches for organic chemicals (Virtual Only)

3.05.V-01 Freely Dissolved Concentration Analysis of Cationic Surfactant Benzalkonium in Sediment Toxicity Test Using Passive Sampling Method

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A cationic surfactant benzalkonium chloride (BAC) has been used in household stuff such as antimicrobials, detergents, and fabric softeners. The impact of BAC on the environment has been of concern in ecotoxicological and environmental studies in recent

years because of its relatively high toxicity and strong adsorption property to negatively charged surfaces (e.g. soils and sediments). For assessing the toxicity of a chemical in sediments to benthic organisms, the freely dissolved concentration (C_{free}) in pore water is regarded as a key factor. C_{free} is usually measured using passive samplers such as solid phase microextraction (SPME) fibers. However, passive sampling methods have not been widely applied to C_{free} determination of cationic surfactants in sediments yet, and the suitability of C_{free} as a measure of exposure to cationic surfactants in sediments is still not clear. In this study, we measured C_{free} of benzyldimethyldodecylammonium (C12-BAC) in sediment toxicity test systems with a passive sampling method using polyacrylate (PA) coated glass fibers and compared the C_{free} with the total dissolved concentration (C_{diss}) in pore water collected by centrifugation. The organic carbon/water partition coefficient ($\log K_{\text{oc}}$) of C12-BAC estimated in passive sampling tests using a river sediment was 6.55 ± 0.04 , comparable to literature values. The results from the preliminary sediment toxicity tests using an amphipod *Hyalella azteca* as test organisms show that $\log K_{\text{oc}}$ of C12-BAC in OECD sediments was 5.25 ± 0.24 , and that the C_{free} in pore water was ca 10 times higher than the C_{free} in overlying water, partially as a consequence of a semi-flow through exchange with fresh water. In addition, C_{free} in pore water was 2–25 times lower than C_{diss} , which may be explained by significant binding of C12-BAC to dissolved organic carbon in pore water. These results indicate that C_{free} measurement can illustrate the distribution behavior of cationic surfactants in sediment test systems and suggest that pore water C_{free} has the possibility to be as a measure of the toxicity of cationic surfactants in sediments.

3.05.V-02 Method Validation for the Determination of Pesticides in Human Blood by Laminar Flow Mass Spectrometry

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Discussions about pesticides focus on the risks they pose to health and the environment. Many studies are being carried out to better understand the impact of pesticides on health, often by establishing a link between the area of use and the proximity to the individual. However, little correlation has been established between exposure to pesticides (through the environment or food) and the presence of residue in the body. Often this study is performed on urine samples which may be distorted by diuresis or issues with the shelf life of pesticides. We have chosen to analyze the pesticides on a serum matrix on a normal population regardless of environmental exposure, focusing on the pesticides most found in our food and the most regulated according to EFSA to ensure high safety for consumers. The objective of the study is to validate an LCMSMS analysis including the QuEChERS sample extraction and cleaning method and to obtain an accurate, reliable, and robust quantification of 19 targeted pesticides using a new technology LCMSMS. This analysis makes it possible to supplement existing epidemiological analysis and to observe a correlation between the exposure of a population to a pesticide, its detection and its persistence in the human body. The 19 molecules chosen are the most found in analyses of pesticides in food and water. Some molecules analysed: Carbendazim, Chlorpropham, Chlorpyrifos, Piperonyl Butoxide, Atrazine The goal was to find a single extraction method to dose these 19 pesticides simultaneously and provide the patient with a comprehensive report on his exposure to pesticides. The LCMSMS method and the use of the QuEChERS extraction was chosen because of its sensitivity and the existence of a single method allowing simultaneous identification and quantification of the 19 molecules Performance to be achieved:- Recovery on a control at 2 µg/L: 70-130% - S/N ratio for qualification transition >3- Limits of quantification: between 0,5µg/L and 10µg/L depending on the molecules- Variability of the internal standard in control solutions and patients: < 3 SD- Repeatability: < 20% or < 10% depending on the concentrations and RSD< 20% on internal standard pic area- Intermediate precision for 30 days: RSD< 20% on concentrations and peak area of Internal Standard and molecules Inter component contamination< 1%

3.05.V-03 Post-Acquisition Data Mining for Fungicides and Their Metabolites in an Inter-City Study Using Ultra-Performance Liquid Chromatography Coupled With High Resolution Tandem Quadrupole Time-Of-Flight Mass Spectrometry

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The presence of antifungal agents in the aquatic environment is a concern in many countries because antifungals are widely used as human pharmaceuticals, in household products and in agriculture. Fungicides are widely detected in wastewater treatment plants, sludge and river surface waters. Antifungals are found in surface waters and wastewater at up to $1 \mu\text{g/L}$ levels. The purpose of this study was to develop an analytical framework utilising retrospective data mining and full quantification with ultra performance liquid chromatography coupled with high resolution QTOF mass spectrometry for selective and sensitive multi-residue analysis of antifungals. The linearity of calibration curves (r^2) were generally > 0.997 for the concentration range 0-1000 ngmL⁻¹. The accuracy in the same day and different three days was typically within the range 83–117% and showed precision below 23% RSD. The SPE recoveries of most of antifungal agents were between 80-119%. However, some compounds provided low or high recoveries due to matrix effect. This method provided low (ng/L) MQLs for most analytes and was applied to investigate their usage and fate of antifungals in catchment located in South-West England.

3.05.V-04 Quantification of Polyaromatic Hydrocarbons (PAH) by Online Spe-Lc/Ms/Ms in Drinking WATER

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Regulatory water directives from around the globe have implemented environmental quality standards for drinking water and in particular in the European Union, they have defined a list of more than 30 priority substances that have been determined to be high risk to humans (1). One class of compounds included are the polyaromatic hydrocarbons (PAH) that are of concern because of their potential adverse effects to our health (2). In the present work we demonstrate a robust and highly sensitive method using online SPE-LC/MS/MS to quantify the five PAHs (Benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, indeno(1,2,3-cd) pyrene) listed in the latest EU drinking water directive (1). The goal of the present work is

to show an alternative to current methods such as LC-FLD (Fluorescence) or even GC/MS in analyzing the regulated PAHs and still meet the strict limits set forth. Moreover, this method needs no further offline sample preparation, because we used PerkinElmer's QSight SP50 Online SPE System. This module in addition to the UHPLC facilitates sample clean-up, enrichment and concentration, obviating the need for elaborate and time-consuming sample preparation procedures. A good example of how we could reach the required sensitivity was with Benzo(a)pyrene, the PAH with the lowest required amount. We could reach 0.002 µg/L, five times lower than the required limit of 0.01 µg/L. Additionally, with the other four PAHs, we could also reach levels of 0.002 µg/L, where the regulation states 0.1 µg/L as a sum of the four. This also easily meets the sensitivity requirements with some room to spare. References: 1. EU COUNCIL DIRECTIVE 98/83/EC [cited 2020 Dec. 10]. Available: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31998L0083&from=EN> 2. CDC Polycyclic Aromatic Hydrocarbons (PAHs) Factsheet [cited 2021 Dec. 01] Available: https://www.cdc.gov/biomonitoring/PAHs_FactSheet.html

3.05.V-05 Seasonal Occurrence and Risk Assessment of Endocrine-Disrupting Compounds in Tagus River Estuary Biota Antía Lestido¹, Mateus Petrarca², Jose Fernandes³, Isa Marmelo⁴, António Marques⁵ and Sara Cunha⁶, (1)Faculty of Pharmacy, University of Santiago de Compostela, Spain, (2)LAQV-REQUIMTE, Portugal, (3)REQUIMTELAQV, Portugal, (4)IPMA, I.P., Portugal, (5)Portuguese Institute of Sea and Atmosphere IPMA, Portugal, (6)LAQV-REQUIMTE, Porto, Portugal

Water pollution in our seas and rivers has a direct impact in flora and fauna and, consequently, on human health. One essential aspect in food safety is to determine health risks associated with the presence of chemical contaminants in food. Such contamination includes, for example, bisphenols from industrial sources, pesticides from crop treatments, or personal care products, which are synthetic organic chemicals frequently used in cosmetic and hygienic products, such as musk fragrances, disinfectant, surfactants and ultraviolet (UV) filters, among others. Most compounds can be classified as endocrine disrupting chemicals (EDC). In this study, the simultaneous analysis of four types of EDC (21 pesticides, 4 polycyclic musk fragrances, 4 UV-filters and 7 bisphenols) in 296 samples including fish, bivalves, crustaceans, earthworm and macroalgae samples collected in Tagus River estuary biota to assess the environmental contamination and dietary exposure to these chemicals. Samples were collected in different seasons (winter, spring, summer, and autumn). In addition, the risk assessment for humans were evaluated. The analysis was achieved by Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) extraction combined with dispersive liquid-liquid microextraction-derivatization, followed by gas chromatography-single quadrupole mass spectrometry (GC-MS). A total of 14 of the 36 chemical compounds analysed could be identified and quantified in the studied biota, including 5 pesticides (alachlor, ethion, DDT, bifenthrin and γ -chlordane), 2 musk fragrances (galaxolide and tonalide), 4 UV-filters (EHS, IMC, EHMC, BP3), and 3 bisphenols (BPF, BPA and BPB). In general, the highest level of analytes in the different species was found in summer. Estimated dietary intakes (EDI) was calculated using the available consumption data of the Portuguese population obtaining a mean EDI of musks ranged between 0.2-5.9 µg/kg bw/day, 0.3-1.8 µg/kg bw/day for pesticides, 0.3-5.2 µg/kg bw/day for UV-filters and 0.1-3.8 µg/kg bw/day for bisphenols. Among fish liver samples analysed in this study, only residual amounts of galaxolide, tonalide, chasmeran, EHS and BPF were detected. In conclusion, based on the risk assessment performed, it is unlikely that there is a potential health risk because the exposure to these compounds is much lower than the tolerable day intake established. Funding: This work was supported by FEDER (Programa Operacional Competitividade e Internacionalização - COMPETE 2020), from PIDDAC through FCT/MCTES project POCI-01-0145-FEDER-028708, by UIDB/04423/2020 and AgriFood XXI R&D&I project, operation No. NORTE-01-0145-FEDER-000041, co-financed by the European Regional Development Fund (ERDF) through NORTH 2020 (Northern Regional Operational Program 2014/2020).

3.05 Advancing analytical and experimental approaches for organic chemicals (Poster)

3.05.P-Tu107 RAINBOWflow CHIP: Ensuring Stable Chemical Concentrations for Toxicity Assessment by a Miniaturised Flow-Through System With Fish Cells

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Fish form an integral part of aquatic ecosystems, and as such comprise a compulsory constituent of toxicity testing for chemical risk assessment and effluent testing; millions of fish are sacrificed annually in this context. One *in vitro* alternative is the use of fish cells. Cell lines of different species and organs exist, and have shown good correlation with *in vivo* results. In the RAINBOW_{FLOW} CHIP project, we use an intestinal cell line of the rainbow trout (*Oncorhynchus mykiss*), RTgutGC, for impedance sensing: cells are seeded on a microfluidic electrode chip and their adherence creates a resistance to the electric current flow, which reflects the health status of the cells. A decrease in resistance is an indicator for loss of cell viability as can be elicited, for example, by exposure to chemicals. Impedance sensing is non-invasive and can be measured in real-time, allowing for time-resolved analysis of cell viability. The aim of this project is thus to employ impedance sensing for chemical hazard assessment. A multi-channel microfluidic setup allows several concentrations to be tested in parallel by flow-through dosing. The flow of medium creates a shear stress which is a positive stimulus for these epithelial cells. In addition, the flow-through dosing allows for constant replenishment of the test substance. This is especially important for difficult-to-test (i.e., volatile and hydrophobic) chemicals, which are prone to losses by sorption and evaporation, thus potentially leading to underestimation of toxicity. Our first results show that stable exposure concentrations could be maintained for three model substances with different physico-chemical properties for at least 24 hours. In the next step, RTgutGC cells will be exposed to these substances for time- and concentration-dependent toxicity testing. Overall, the RAINBOW_{FLOW} CHIP biosensor will allow the investigation of toxicity of chemicals with stable or purposefully fluctuating concentrations over time without the need to conduct animal experiments. Moreover, extensions of this setup – specifically for applications to on-line effluent monitoring in the field – are also currently being implemented.

3.05.P-Tu108 Hydrophobic Organic Chemicals in Sediment Toxicity Tests - Optimizing Passive Dosing by 3D Printing Techniques

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Ecotoxicological tests of hydrophobic organic chemicals (HOC) come with different challenges. HOC tend to strongly sorb to organic material in the test system as well as to test vessels, materials and/or test organisms which complicates the exposure control of the freely dissolved concentration and might finally lead to an underestimation of the measured effects. In sediment toxicity testing the high amount of organic material can result in strong sorption effects, and a freely dissolved pore-water concentration (effective concentration) much lower than the total concentration. Also, analysis of the pore-water concentration is not straightforward, but information about the distribution of the chemicals and the resulting pore-water concentration is crucial for the ecotoxicological assessment of HOC in sediment toxicity studies. Passive dosing (PD) is a method that offers a reliable exposure control and leads to a constant dissolved concentration of the test compound over time. By applying an inert reservoir to the test system, loaded with the HOC, PD can make up for any losses of the test compound over the course of the experiment. PD reservoirs are often made of silicone and can have different shapes such as O-rings, tubes, or sheets. However, in a sediment toxicity test, the use of these forms is problematic due to their inhomogeneous distribution in the sediment. Here, a more complex form with a high surface-area-to-volume ratio was selected to ensure a homogenous distribution throughout the sediment layer and fast release kinetics. The design of the new passive dosing device was developed *in silico* in an adaptive manner and produced by 3D printing a casting form using a polylactic acid (PLA) filament. The used PLA is transparent, biocompatible, and food-safe to ensure no contamination of the silicone from colours or other additives. The 3D printed PLA negative form was finally casted with a food-grade 2-component silicone to create the final device. The distribution of selected hydrophobic model compounds in standard sediments was tested as well as their release kinetics from the new silicone forms. Furthermore, we will report the distribution coefficients between silicone, water and sediment which allow to estimate the pore-water concentration.

3.05.P-Tu110 Bioaccessibility of Persistent Organic Pollutants From Indoor Dust in Human Gastrointestinal Tract

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Indoor dust ingestion is considered one of the main human exposure routes for many chemicals, particularly consumer product additives such as flame retardants and plasticizers. Bioaccessibility of chemicals from ingested dust has been defined as the fraction of a compound that is released from its matrix within the gastrointestinal tract and thus becomes available for intestinal absorption. Bioaccessibility in terms of ingestion and risk assessment is frequently discussed and the understanding of the process for individual chemicals can help to improve risk assessment for human health. For this purpose, numerous tests have been developed which simulate transfer from the matrix to the gastric or intestinal fluids. One commonly used test is the modified physiologically based extraction test (PBET), which simulates processes in the stomach (for one hour) and small intestine (for four hours). In this study, the bioaccessibility of a set of chemicals of concern (halogenated flame retardants, polychlorinated biphenyls, organochlorine pesticides) from dust samples was tested with PBET. Dust samples were collected in seven microenvironments: (1) pre-fab apartment blocks, (2) new houses built after 2005, (3) kindergartens, (4) schools, (5) offices, (6) public spaces, and (7) cars. The finest fraction (< 0.25 mm) of the dust was used because it is the most relevant fraction for ingestion. The bioaccessible fraction of chemicals of concern varied based on dust origin (e.g., higher bioaccessibility of some compounds in public spaces) as well as on the chemical itself. Bioaccessible fractions were mostly under 10 % of total concentrations extracted by organic solvents, which suggests using total dust concentrations overestimates the internal exposure of pollutants from dust, e.g., when performing risk assessment. Supported by the grant 19-20479S from Grant Agency of the Czech Republic.

3.05.P-Tu111 Evaluation of Different Solid-Phase Extraction Methods for Effect-Based Assessment of Hazardous Chemicals in WATER - Differences in Recovery and Background Activities

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Solid-phase extraction (SPE) is commonly used for enrichment of different water samples (e.g. drinking water, wastewater and surface water) as preparation for effect-based assessment of hazardous chemicals in the sample. There is a wide range of sorbent materials available for solid-phase extraction, capturing different classes of pollutants. Further, different sorbents can be combined in multi-layer columns. It is well-known that environmental and drinking water samples can be polluted with a very complex mixture of chemicals. Effect-based water quality assessments are commonly run in a battery of bioassays, with each a specificity to one endpoint. As water samples can be contaminated with a very complex mixture of chemical pollutants, it is desirable to use SPE sorbents that can capture as broad range of pollutants as possible, to gain a holistic understanding of the hazardous chemicals in a sample and potential cocktail effects. At the same time, it is of utmost importance to ensure that the sorbent material is not contaminating the sample with any compounds that might interfere with the effect-based assays and cause false positive results. In this study, we have evaluated three commercially available SPE columns as well as one custom-built SPE column to observe the recovery of bioactivity of hazardous chemicals in both environmental water samples as well as spiked pure water. Additionally, we evaluated the potential contamination of water samples from sorbent materials by processing pure water controls. The extracts were then assessed in a battery of commonly applied bioassays for water quality assessment. Endpoints as xenobiotic metabolism,

hormone-receptor mediated effect as well as adaptive stress response were included. The results show differences in recovery between the sorbent materials and also indicate that some sorbent materials might contaminate samples with bioactive compounds which might interfere with the bioanalysis. It is evident that the choice of sorbent will greatly affect the outcome of the bioassay.

3.05.P-Tu112 Chemical Activity As a Quantitative Measure to Assess the Effects of Contaminant Mixtures on Phytoplankton

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Phytoplankton cells accumulate organic contaminants from the water phase through passive partitioning. The accumulating rate of these compounds is modulated by the physicochemical properties of both contaminants and phytoplankton cells. Understanding the mechanism that regulates phytoplankton's sensitivity towards a mixture of organic contaminants will better predict the effects of chemicals on biodiversity and productivity in aquatic ecosystems. Previous studies demonstrated the applicability of the chemical activity concept for different organisms; however, there is a lack of studies on primary producers. Our aim is to assess (eco)toxicological responses to chemical activities ranging from 0.01 to 0.2 of mixtures of four PAHs in the model phytoplankton species *Rhodomonas salina* (KAC 30). The concentration of each chemical is maintained below its established no effect concentration (NOEC) for any specific toxicity mechanism. A passive dosing method is used to obtain stable exposure levels. The concentrations in both water and biota are measured with GC-MS methods to confirm actual exposure levels to the chemicals. Growth and yield inhibition, membrane integrity, and photosynthetic activity will be assessed. We hypothesize that the chemical activity in the mixture and the observed effects in *Rhodomonas salina* follow a dose-response curve and that membrane integrity stands out as a sensitive and valuable early-warning signal to catch PAH-baseline toxicity.

3.05.P-Tu113 Questioning the Appropriateness of Sieving for Processing Indoor Settled Dust Samples

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Indoor settled dust is a common matrix for assessing human exposure to organic chemicals of concern (e.g., flame retardants, plasticizers). However, many of the techniques commonly used to collect and process indoor dust samples are not standardized, and are based on techniques adapted from other matrices. In this study, we consider the implications of sieving for pre-processing dust samples and the impact of this technique on measurements of chemicals in dust. Using composite dust samples from seven different microenvironments (pre-fab apartment blocks, new homes, kindergartens, schools, offices, public spaces, and cars), we investigated the effect of sieving by comparing individual size fractions of dust (1-2 mm, 0.5-1 mm, 0.25-0.5 mm, < 0.25 mm). Analyses of polycyclic aromatic hydrocarbons (PAHs) in dust fractions was combined with carbon analysis and optical microscopy to better understand the impact of sieving on observed distributions. The technique of sieving to pre-process dust samples developed from soil science, and is appropriate for dry, non-fibrous particles. However, microscopy analysis suggests that dust is not effectively separated by size fraction with this technique. Dust forms aggregates, which contain fine particles and are retained by coarser sieves, particularly due to the content of fibres, which are not found in e.g., soils. Thus, fine dust fractions will be biased towards non-fibrous particles, and coarse dust fractions will contain substantial fine particles. This was supported by measurements of carbon (total and organic) in individual size fractions of dust, which showed a high contribution of organic carbon (21±10%) across all dust size fractions without systematic variation with particle size. PAHs, rather than being predominantly found in the finest fractions, also had no systematic increase with finer size fractions, unlike typical distributions in aerosols or soils, where finest fractions also contain the highest levels of lipophilic organic compounds. This observed heterogeneity, attributed to the formation of aggregates during vacuuming and sieving, has implications for how dust samples are collected and processed for the purpose of exposure assessment. We emphasize the importance of carefully questioning techniques adapted from other matrices to understand how new factors (e.g., fibrous dust particles) can impact study outcomes. This work was supported by grant 19-20479S from the Grant Agency of the Czech Republic.

3.05.P-Tu114 Comparing Non-Invasive Passive Sampling Approaches to Monitor Human Exposure to Environmental Contaminants

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Around the globe, human health is affected by exposure to a wide range of harmful xenobiotics from diverse sources and uptake routes. Emerging chemicals, compounds generated as industrial byproducts and also legacy contaminants are of concern. Monitoring human exposure to these pollutants, however, relies heavily on invasive sampling methods. Non-invasive sampling approaches using silicone passive samplers have been developed to replace these invasive methods for large-scale monitoring studies. This project aims at the comparison of passive sampling approaches designed to cover different exposure routes. Silicone wristbands, which sample airborne, but also dermally excreted compounds are compared to silicone skin patches that have a larger contact area to facilitate sampling of the excreted internal contaminant burden of study participants. Furthermore, the skin patches are shielded against airborne particle deposition by an isolation layer and are worn on the upper thigh, hence usually covered by clothes. The contaminant-blocking capability of different materials for such isolation layer was tested in "sandwich" partitioning tests. Fabric layers, creating a passive, physical barrier, and activated carbon textiles that can trap compounds before they reach

the silicone sampler, were placed between a donor silicone disk spiked with model contaminants, and the clean receptor silicone disk. The results of a partitioning trial lasting 5 days (i.e., the intended sampling time) showed that for most hydrophobic contaminants, a simple gauze layer sufficiently slowed down the mass transfer between donor and receptor to substantially reduce the impact of airborne particles on silicone skin patches. Low molecular weight PAHs and more hydrophilic substances could still partly penetrate this isolation layer in a wet environment (emulating sweat). This penetration could be prevented to some extent by an added activated carbon layer. However, the benefits were considered too small to make up for the added discomfort to participants and increased challenges during the chemical analyses of samplers containing activated carbon particle traces.

3.05.P-Tu115 Passive Sampling to Detect Resuspension-Induced Increases in Dissolved WATER Concentrations of HOCs *Inna Elina Nybom¹, Ursula Strandberg¹, Clare Bradshaw², Cynthia de Wit³, Annika Jahnke⁴ and Hans Peter Arp⁵, (1)University of Eastern Finland, Finland, (2)Stockholm University, Sweden, (3)Stockholm University, Stockholm, Sweden, (4)Helmholtz Centre for Environmental Research - UFZ GmbH, Germany, (5)NGI, Norway*

Sediments near urban and industrial centers are historically contaminated with diverse environmental pollutants. Resuspension of these sediments, e.g. during construction activities, leads to an increase of contaminated sediment particles in the water, which consequently can drive an increase in freely dissolved water concentrations of sediment-associated pollutants. Low-density polyethylene (LDPE) passive samplers were applied to measure the freely dissolved water concentrations of hydrophobic organic contaminants (HOCs) in the water column during a large-scale bridge construction work, known to induce sediment resuspension. Passive samplers were deployed at different water depths; 1m from the bottom, middle of the water column and 1m from the surface. Two sampling campaigns were executed, over the period of 3-6 weeks before the construction work (May-July) and over 6 weeks during the construction (September-October). Despite the relatively short sampling time, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) from the water column were detected, and their freely dissolved water concentrations were derived. The changes over time in the freely dissolved concentrations were demonstrated with the applied sampling technique. Resuspension induced increase in water concentrations was observed specifically near the sediment surface for analytes with $\log K_{OW}$ 5-6. Factor of 1.7-4.6 increase in the dissolved water concentrations was observed during the sediment resuspension event compared to the previous deployment. Measurements at the reference site, beyond the influence of the construction activities, demonstrated seasonal variation in the dissolved water concentrations. For analytes with $\log K_{OW}$ 5-6, a factor of 0.7-1.7 difference in water concentrations was observed between the two sampling times near the sediment surface. However, the influence of the sediment resuspension at the construction site on the freely dissolved concentrations was evident and up to a factor of 3 higher than the observed seasonal variation.

3.05.P-Tu116 Applying Solid Phase Microextraction to Determine Biodegradation Kinetics of Chemical Mixtures in WWTP Sludge

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Biodegradation tests are traditionally designed for single chemicals. Testing of chemicals in mixtures and tracking the degradation of each chemical has the advantages that it improves the internal comparability of biodegradation data and their environmental relevance. Recently, we developed a platform for biodegradation testing of hydrophobic and volatile chemicals. The biodegradation and the analytical step were aligned by using headspace vials as test systems and automated Solid Phase Microextraction (SPME) coupled to GC-MS to measure substrate depletion relative to abiotic controls. The platform works well for biodegradation testing in lake, sea and river water that all have a limited suspended solids (SS) content. This study was directed at extending the applicability domain of the approach to media with higher SS content such as WWTP sludge. Activated sludge from a Danish wastewater treatment plant were filtered using (1) 0.5 mm sieve, (2) 0.125 mm sieve and (3) 25 μ m filter. The first filtration removed larger particles, the second reduced SS content from 2.4 g/L to 0.25 g/L, the third reduced SS to 0.0027 g/L. Abiotic test systems were prepared with autoclaved sludge and 0.1% NaN_3 . 13 test chemicals in methanol (fragrances, UV filters, plasticizer, PAHs) were added to 240 mL test systems by microvolume spiking (5 μ L) for μ g/L concentrations. Test systems were incubated at 12°C and subsampled in time-series covering 17-115 min, 1-48 hours or 1-29 days. Samples were diluted 1:1 in ethanol and stored at -18°C. The ethanol conserved the sample, avoided breakage of vials due to ice formation, and extracted chemicals from the sludge. This solution was filtered (0.2 μ m) and diluted in pure water to ~10% ethanol concentration before analysis using SPME. DNA extraction and microbial community analysis were performed on the three inocula and after 48 hours incubation with/without test chemicals. A markedly different microbial composition was seen in the 0.0027 g SS/L filtrate compared to the 0.25 g SS/L and 2.4 g SS/L sludge, for which minor differences were observed. Incubation for 2 days did not affect the microbial composition. Linalool, naphthalene, citronellol, α -isomethylionone, phenanthrene, and 2-ethylhexyl 4-methoxycinnamate degraded within 48 hours in the 0.25 g SS/L and 2.4 g SS/L sludge. Drometrizole, dicyclohexyl phthalate, homosalate and anthraquinone required longer incubation. Advantages and disadvantages of the test method will be discussed.

3.05.P-Tu117 Effects of Algae and Fungicides on the Fate of a Sulfonylurea Herbicide in a Water-Sediment System

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The impact of pesticide mixtures on various soil parameters has been extensively studied, whereas research on effects in the

aquatic environment is scarce. Furthermore, investigations on the consequences of chemical mixtures on the biodegradation kinetics of parent compounds remain deficient. Our research intended to evaluate potential effects by combined application of an agriculturally employed tank mixture to aquatic sediment systems under controlled laboratory conditions. The mixture contained two fungicides and one radiolabelled herbicide of which the route and rate of degradation was followed. One set of aquatic sediment samples was incubated in the dark. A second set of samples was controlled under identical conditions, except for being continuously irradiated to promote algal growth. In addition, the algal biomass in irradiated aquatic sediment was monitored to determine its effects and a potential role in the biodegradation of iodosulfuron-methyl-sodium. The study results showed that the herbicide, although hydro- and photolytically stable throughout the experiment, metabolised faster (DT50 1.1 to 1.2-fold and DT90 2.8 to 4.5-fold) when continuously irradiated in comparison to dark aquatic sediment. Both fungicides had a significant prolonging effect on the biodegradation rate of the herbicide. In the presence of fungicides, DT90 values increased 1.5-fold in the irradiated, and 2.5-fold in the dark systems. Additionally, algae may have influenced the metabolism of the herbicide in the irradiated systems, where shorter DT90 values were evaluated. Even so, the algal influence was concluded to be indirect.

3.05.P-Tu118 Collision Cross Section (CCS) Value As an Additional Identification Point for Chemical Characterization: Development of a Lc-Esi-Tims-Qtofms Database for Environmental (Bio)monitoring Studies

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Thousands of chemicals are estimated to be dispersed throughout the environment and may pose a threat to ecosystems and human health. Exposure to chemicals can be assessed by the monitoring of environmental contaminants in the ambient environment (air, water, soil), in organisms from various trophic levels, and in human biological samples (blood, urine). The increased availability of high resolution mass spectrometric (HRMS) techniques in the chemical analysis allows the wide-scope screening of complex samples. The hyphenation of ion mobility spectrometry (IMS) with HRMS has come to add a new dimension for compound separation and identification; namely collision cross section (CCS). This additional dimension enhances the identification confidence in target screening. To date, very few studies are available in the literature providing integrated LC-IMS-HRMS databases of organic contaminants. Here, a database containing more than 1,000 environmental contaminants (pharmaceuticals, illicit drugs, pesticides, industrial chemicals, etc.) was built by utilizing liquid chromatography coupled to electrospray ionization—quadrupole-time-of-flight mass spectrometry equipped with trapped ion mobility spectrometry (LC-ESI-TIMS-QTOFMS). The overall aim of this study was the establishment of a CCS-aware database for wide-scope target screening. For the database development, a quality control protocol was followed to ensure high-quality CCS measurements based on the guidelines for the Unified CCS Compendium. Accuracy was assessed by analyzing a standard solution with reference CCS values before, during and after the analyses, and the individual CCS error was found to be below 1%. To assess CCS values reproducibility, all solutions were analyzed in triplicate resulting in $\%CCS < 0.7\%$ being achieved in most cases. Therefore, beyond molecular formula (accurate mass, isotopic pattern), retention time, and qualifier ions (adducts, fragments etc.) information for each compound, the developed database was enriched with ion mobility derived CCS values. To the best of our knowledge, this is the first study reporting such an extensive dataset incorporating CCS values for a broad range of environmentally relevant compounds. Wide-scope target screening with the developed database was applied in different samples. Selected examples unveiling the performance of adding another dimension for analyte separation and sample characterization are presented.

3.05.P-Tu119 Advancing Electron Spin Resonance (ESR) Spectroscopy of Spin Labelled Organic Compounds in Environmental Chemistry

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Electron spin resonance (ESR) spectroscopy has successfully been applied since many years for investigating the structure and dynamics of biological macromolecules and assemblies, particularly membranes and proteins. However, its potential for investigating the various abiotic and biotic interaction processes in soil or sediment has not yet fully been utilized. ESR spectroscopy of tailored organic compounds labelled with stable paramagnetic nitroxides is a non-destructive method, which delivers direct information of their interaction processes with the soil or sediment environment on a molecular scale. Strong and weak adsorption, covalent binding and physical entrapment can be distinguished *in situ*. Moreover, the accessibility of immobilized spin labelled compounds for antioxidant such as sodium ascorbate and the polarity in the close vicinity of the nitroxide can be investigated [1]. The line shape of the continuous wave (cw) ESR spectrum reflects the reorientational motion of the nitroxide spin label, which in case of restricted Brownian motion can be characterized by its rotational correlation time, anisotropy, and (angular) amplitude. The rotational correlation time is a measure of the duration over which molecules maintain a particular orientation before random thermal motion reorients them. Cw ESR at different frequencies (9 – 95 GHz) spans the sensitivity for rotational correlation times from picoseconds to hundreds of nanoseconds [1]. Weak restrictions of the nitroxide reorientation, as e.g. found for small spin labels dissolved in water, result in nearly isotropic reorientational motion with correlation times less than 1 ns. Restriction of the nitroxide reorientational motion upon binding or sorption results in the decrease, e.g., of the rate or amplitude of the reorientation due to sterical hindrance yielding increased apparent hyperfine splitting and line widths. Time resolved investigations of spin labelled aromatic amines (aniline, sulfadiazine) with humic acid in solution and with model clay and soil suspensions demonstrate the potential of ESR spectroscopy for elucidating the interaction processes of organic compounds with soil and sediment (see platform presentation Ricke et. al.). References: [1] Klare JP, Steinhoff H-J in:

3.05.P-Tu120 Interaction Kinetics and Accessibility of Sulfadiazine in Model Clay-Humic Acid Suspension: Electron Spin Resonance Investigations With Nitroxide Spin Label

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Electron spin resonance (ESR) spectroscopy of spin-labelled organic compounds is a microscale technique for the investigation of interaction processes in soil and sediment (see poster Steinhoff et al.). In the present work, the interaction of model clay-leonardite humic acid (LHA) suspensions with nitroxide spin-labelled antibiotic sulfadiazine (SL-SDZ) was studied. The objectives of our work are to (1) distinguish between adsorbed, entrapped and covalently bound species; (2) investigate the influence of the clay mineral on the kinetics of immobilization of SL-SDZ in model clay-LHA suspensions; (3) determine the accessibility of SL-SDZ in model clay-LHA suspensions important for remobilization and bioavailability. SL-SDZ and its spin labelled metabolite SL-N-acetyl-SDZ were synthesized. LHA was incubated with laccase to enhance the amount of reactive quinone groups of LHA and then mixed with the smectite mineral clay Ca-hectorite. The Ca-hectorite-LHA suspension was incubated with SL-SDZ or with SL-N-acetyl-SDZ, which is not susceptible for covalent binding of the aniline moiety to LHA. Upon incubation of SL-SDZ with the Ca-hectorite-LHA suspension ESR spectra were recorded in short time intervals over 46 d. The three sharp hyperfine lines of the free nitroxide decreased with time, whereas the broadened ESR signal, which indicates strong restriction of the reorientational motion of the spin probe, increased simultaneously. The amplitudes of the broadened ESR signal were fitted with an exponential model with pseudo first-order time constant of 82.6 ± 25.0 h for the immobilization of SL-SDZ. A fraction of SL-SDZ was obviously covalently bound to LHA as it was observed without Ca-hectorite, but with a reaction rate 4.7 times slower. An offset indicated a very rapid immobilization of SL-SDZ directly after mixing with the suspension due to strong adsorption to LHA. The fast decrease of the signal of the unbound SL-SDZ corroborates this observation. Ca-hectorite-LHA suspensions were incubated with SL-SDZ or SL-N-acetyl-SDZ. The suspensions were mixed with the hydrophilic antioxidant Na-ascorbate. The fast reduction of the unpaired electron of the immobilized and mobile fractions disproved physical entrapment. Covalent binding of SL-SDZ reduces the accessibility compared to strong adsorption. ESR investigations of spin labelled SDZ revealed unspecific adsorption and covalent binding in model soil suspensions of LHA with Ca-hectorite, but no physical entrapment.

3.05.P-Tu122 MALDI Mass Spectrometry Imaging As a New Tool in Biotic Systems

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The OECD test guidelines for testing of chemicals combine a collection of 150 of the most relevant internationally agreed testing methods used by government, industry and independent laboratories to identify and characterize potential risks of chemicals. Over the last few years, there was a growing interest in developing test guidelines using fish and amphibian embryos, most of them being dedicated to the identification of endocrine active chemicals. Among these, the *Xenopus* Eleutheroembryonic Thyroid Assay (XETA, OECD Test Guideline 248) deliver data used for assessing the capacity of chemicals to activate or inhibit the transcription of a genetic construct thereby allowing the identification of chemical potentially active on thyroid axis. These guidelines, on the one hand, have many common methodological features with those applied for the testing of pharmaceuticals or plant protection products, but information about the distribution in- or outside of specific tissues of tested animals is limited. Matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) provides, *in situ*, label-free molecular and spatial information of a range of compounds like pesticides and endogenous compounds (e.g., hormones, lipids, amino acids). The combination of morphological data with mass spectrometric data leads to a new tool for a better understanding of the behavior of test items in biotic model systems. *X. laevis* was selected as the representative test organism and exposed according to OECD TG 248. First MS imaging experiments of selected animal tissues were carried out on a Q-Exactive™ Hybrid-Quadrupole Orbitrap mass spectrometer, coupled to an AP-SMALDI5AF ion source. Mass spectra with high mass accuracy and high mass resolution were acquired for the precise identification of target molecules by the detection of monoisotopic masses of interest. Measurements were performed with a high spatial resolution of 30 μ m step size to distinguish the distribution of selected test items in particular areas of interest. *X. laevis* cryo-sections (25 μ m thickness) were optically assessed after optimization of the sample preparation protocol and characteristic anatomical structures (eyes, brain, intestine, tail) were identified. The test items in *X. laevis* were traceable by MALDI MSI and revealed a particular distribution in different sections of the animals and allowed a better understanding into the mechanistic behavior of the test item in the XETA.

3.05.P-Tu123 Polychlorinated Biphenyls in Mine Water - Miniaturized Approaches for Congener Specific Analysis and Palladium Catalyzed Dechlorination

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Polychlorinated biphenyls (PCBs) are among the persistent organic pollutants (POPs) that have been banned worldwide since 2001, due to their persistence, bioaccumulation and chronic toxicity even in very low concentrations. Produced as industrial chemicals, PCBs were used as insulating oil in transformers and capacitors, including underground mining. Due to high salt and metal concentrations, mine water represents one of the largest pollutant streams by volume in the world. However, until today especially inorganic pollutants are considered and treated. But as a legacy of mining, (closed) mines can contain relevant quantities of PCBs. Mine water must be pumped out regularly, and is discharged into rivers. When monitoring of PCB contamination takes place, it is mainly targeted at receiving waters. From the group of PCBs with theoretically 209 different

congeners, only six indicator PCBs and one dioxin-like PCB are considered. The aim of the study was both to determine concentrations of the most relevant PCB congeners in mine waters and to develop a treatment approach to reduce these concentrations. Catalysis promoted by palladium nanoparticles (Pd-NPs) is seen as a promising strategy to efficiently remove reducible contaminants in water treatment approaches. For PCB analysis, a solid-phase microextraction (SPME) method coupled to gas chromatography-mass spectrometry (GC-MS) was optimized for the sensitive and solventless extraction and detection of numerous PCB congeners directly from untreated mine water. Dechlorination experiments were carried out with the main detected PCB congeners. The SPME method was highly suitable for simultaneous extraction of the reactants and dechlorination products directly from miniaturized reaction vessels. As a result, more than 20 PCB congeners were quantified in water from five different mines in Germany. The presented method allows a comprehensive and labor saving analysis of PCBs even in lowest volumes of 10 mL untreated, matrix rich mine waters. Given the detected concentrations and the estimated annual load, action is needed even at very low levels of PCB occurrence due to their persistence and bioaccumulative properties. Hydrodechlorination using Pd-NPs can convert PCBs to the easier biodegradable biphenyl within minutes. Future experiments aim at protecting the Pd-NPs during dechlorination reactions for better stability and continuous usage.

3.05.P-Tu124 Synthesis and Certification of New Standards for Chlorinated Paraffins

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Introduction Chlorinated paraffins (CPs) represent a class of industrial chemicals used as high-temperature lubricants in metal-working machinery. The total global production remains largely unknown but is believed to exceed at least two million metric tonnes per year. Short-chain CPs (SCCPs) have been prohibited in the EU since 2017. Recently the European Chemicals Agency (ECHA) has added the medium chain CPs (MCCPs) to the list of Substances of Very High Concern. The validation of analytical methods for the identification and quantification of chlorinated paraffins in environmental matrices and foodstuff is being hindered due to a lack of certified reference materials (CRMs). As a result, errors in measurements within individual laboratories can occur. The principal goals of the current projects is to develop reference standards with a defined response factors, which can be used to mimic the industrial mixtures. Such reference standards will then be used to certify the SCCP content in a fish CRM offered by the EU Joint Research Council (JRC). **Materials and Methods** CRMs of individual single SCCPs, ¹³C labelled SCCP internal standards were prepared by synthesis. Single chain congener mixtures have been made by UV light catalyzed chlorination. Single individual compounds have been analyzed for chlorine content using an accredited titration method and of NMR and the results are compared with the theoretical values. Individual compounds and the single chain mixtures were analyzed by GC-MS-MS, high resolution GC-MS and by GC-GC.. Water, solvent and ash content was determined by TGA. **Results** To date, we have produced approximately 40 individual single chain CPs, ¹³C-labelled individual CPs, and 10 single chain CP congener mixtures. Further single component mixtures will be prepared for quantification together with defined mixtures and single chain mixtures. The chemical purity is established by one or several of the following GC-based methods: GC-FID/MS, GC-MS-MS, high resolution GC-MS and GC-GC-MS. Various NMR techniques were developed for chlorine content determination and are compared with theoretical values for single components. **Conclusions** We have prepared a range of CP reference standards and these will be used to prepare the CP CRMs urgently required to support reliable analytical methodologies. These materials will be characterized by a combination of certified analytical methods including purity determination by GC-FID, identity by NMR and excess water, solvent and ash by TGA in addition to stability and homogeneity assessments.

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3.05.P-Tu125 The VANDALF Project: Linking Chemical Detection and Toxicological Endpoints From Wastewater

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In Europe alone, more than 70.000 different chemicals are used and many of these enter the wastewater system on daily basis. Conventional wastewater treatment plants (WWTPs) are not designed to remove complex mixtures of organic chemicals present at trace concentrations ($\mu\text{g L}^{-1}$ to ng L^{-1}) in wastewater, and only a minor part of total toxicity (1-5%) from wastewater was prior explained by chemicals included in target screening. Hence, there is an increasing necessity to combine analytical and bioanalytical tools with the potential to address and identify unknown chemicals posing a risk to the aquatic environment. In the ongoing project VANDALF, the goal is to develop tools to identify chemicals of emerging concern (CECs) in wastewater. An overall workflow was established, including representative sampling of wastewater from several WWTPs at different timepoints and state of the art sample preparation techniques to prepare wastewater samples for further chemical and toxicological investigations. The sample extracts were analyzed on several chromatographic platforms for chemical composition using both target, suspect, and non-target screening methods. Concurrently, these samples were screened for toxicity using both *in vivo* and *in vitro* assays. Multivariate approaches are then used to identify the chemical fingerprints contributing to observed toxic effects. Via the analysis of both influent and effluent wastewater samples, it is also possible to screen for persistent compounds and evaluate removal rates through the different treatment steps of the WWTP. In addition, we can also address formation of metabolites and pinpoint CECs to include in future monitoring and regulatory measures. At the SETAC poster session specific

chemical and toxicological results will be presented: Our present results agree with previous studies on toxicity from target compounds. This was especially the case for estrogen agonist and aryl hydrocarbon reporter gene assays. However, we were able to explain a larger contribution from target compounds on toxicity in an algae growth assay. With the use of suspect and non-target screening in VANDALF, there is a potential to identify more toxicity due to identification of additional compounds. Additionally, certain trends were observed in chemical diversity (e.g. anti-psychotics and drugs of abuse) as well as trends in the concentration of chemicals (e.g. beta blockers) between WWTPs at different locations and regions of Denmark.

3.05.P-Tu126 Trace-Level Analysis of Organic Pollutants in Atmospheric Particulate Matter by GC Q-Exactive Orbitrap MS

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Over the past decade, gas chromatography coupled to Q-exactive orbitrap mass spectrometry (GC-Orbitrap-MS; Thermo Fisher) was developed, offering compound identification and high-resolution mass-accuracy analysis [1]. GC-Orbitrap-MS (Thermo Fisher) was tested with standard reference material (SRM2260a) and organic tracer compounds, e.g. traffic and wood burning emissions, in ambient air PM_{2.5} in full scan mode and compared to conventional GCMS analysis. GC-Orbitrap-MS analysis of polycyclic aromatic hydrocarbons (PAHs) in standard reference material (SRM2260a) and organic tracer compounds in ambient air particulate matter (PM_{2.5}) gave very low instrumental uncertainties (1% - 15%) and high linearity over a wide concentration range (0.5 pg and 500 pg/ μ L). Good reproducibility was obtained with the GC-Orbitrap-MS compared to conventional GCMS method. The detection limit of the GC-Orbitrap-MS method for full scan analysis of PAHs in PM samples was 0.5 pg/ μ L (compared to 5 pg/ μ L in GC-MS method), allowing the analysis of small sample fractions (< 1m³) and/or samples with short sampling times (1.5 h), thus, increasing the temporal and spatial analysis of organic tracer compounds in ambient air PM. Punch samples (\varnothing = 3.4 mm) were taken from PM_{2.5} filter and filter strips of Aethalometer AE51 (AETHLABS). This pocket-sized instrument is designed for real-time Black Carbon monitoring. Analyses of filter strips that collected 0.14 m³ in 24 hours showed levels above the quantification limit for PAHs, among them the regulated and most toxic PAH: benzo[a]pyrene, as well as several organic tracer compounds for source apportionment (Figure 2). The correlations between PAHs and BC was very strong, although independent in the two sites due to their different dominating emission sources (traffic vs. biomass burning). The work we present shows that GC-Orbitrap-MS can be used for routine analysis of organic contaminants in atmospheric PM samples and exposure studies at high precision and accuracy, while saving time and consumables in the analysis [2]. [1] Eliuk S., Makarov A. 2015. Annual Review of Analytical Chemistry. 8, 61-80. [2] van Drooge BL., Prat M. R., Jaén C., Grimalt JO. Submitted. J. Chrom. A.

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3.05.P-Tu127 Study of the Adverse Effects of the Insecticide Clothianidin on the Stingless Bee *Tetragonisca Angustula* L., Based on Toxicity Tests and Preliminary Analysis of Environmental Risk

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The collection of scientific information about bees proves their importance as natural pollinators, not only for agricultural crops, but also for the genetic diversity of plants which, in a macro view, means maintenance the stratum of primary producers in the whole world's ecosystems. The excessive use of pesticides is one of the factors that can compromise the health, growth and survival of hives and bees. The present research focused on the chemical determination of clothianidin, a neonicotinoid, in the tissues of *Tetragonisca angustula* stingless bees by the optimized QuEChERS method, and analysis by UPLC-MS/MS and a C18 column (100 x 2.1 mm, 1.7 μ m). Were obtained chromatograms with 5 minutes runs, recovery percentages of 85.05%, 99.01% and 74.58% at three levels studied, 0.001, 0.01 and 0.05 mgL⁻¹, respectively, with RSD less than 20%. The detection and quantification limits obtained were 0.00077 and 0.00232 mgL⁻¹, respectively. The toxic effects were also studied from the acute oral exposure of adults of *Tetragonisca angustula*, the LD₅₀ was determined and shown to be 0.160 ng a.i./bee after 24 hours of exposure. From the data obtained in these studies, the risk quotient (RQ) was calculated for a preliminary analysis of the environmental risk in cotton crops, as well as the possible risk of product drift, based on the commercial product Zellus® SC. Therefore, the results showed that clothianidin represents a mortality risk for bees. And, together with the chromatographic method, we can confirm and quantify the presence of this pesticide in these insects.

3.05.P-Tu128 Batch Equilibrium Experiments Indicate Weak Temperature Dependence of Soil/Sediment-Water Sorption for Cyclic Volatile Methyl Siloxanes

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The organic carbon normalized partition coefficient, K_{OC} , describes the equilibrium distribution of a chemical between water and particulate organic matter, and it is a key parameter in many chemical fate and transport models. In recent years, numerous studies have been published pertaining to environmentally relevant physicochemical properties of cyclic and linear volatile methyl siloxanes (VMS), including K_{OC} . Studies of the sorption behavior of several VMS substances between water and soil or sediment have been reported, with K_{OC} values determined either directly using the batch equilibrium method, or indirectly from measurements of volatilization rates from particle-water slurries. So far, the temperature dependencies of K_{OC} values for the cyclic VMS compounds octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) have been investigated only by the

indirect purge-and-trap method. The present study examined the K_{OC} temperature dependencies of D4 and D5 by the batch equilibrium method. Notwithstanding unknown variations arising from use of sediments of differing origin, empirical K_{OC} values reported for 25 °C by the different methods agreed to within approximately 0.5 and 0.7 log units for D4 and D5, respectively. However, at lower temperature of 4-5 °C, these differences increased significantly, with batch equilibrium K_{OC} values lower by 1.8 log units for D4 and 1.3 log units for D5. Whereas the K_{OC} values by the purge-and-trap method increased with decreasing temperature, giving sorption enthalpies (ΔH_{OC}) of -79 kJ/mol for D4 and -48 kJ/mol for D5, the new batch equilibrium K_{OC} values showed modest *decreases* at lower temperatures with corresponding ΔH_{OC} values of 0 (i.e., not statistically significant) to +13 kJ/mol. These findings could have significant implications for the predicted fate of D4 and D5 in cooler remote regions particularly. Ongoing work is exploring how experimental differences between the studies, such as sorbent-water ratios or preparation and spiking of sorbents, might influence the observed discrepancies in K_{OC} values. Additionally, the dynamic method is being assessed from a theoretical perspective to identify potential bias arising from assumptions about the behavior of VMS materials in the purge-and-trap system. It is expected that the current study will be an important step toward resolving these apparent discrepancies, and eventually defining consensus values of these important parameters for the VMS substances.

3.05.P-Tu129 Early Life Stage Development and Oxidative Stress Markers in Lake Trout Fry From a Lake Used to Conduct Model Oil Spills

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In Canada, and across the globe, oil production as a source of energy remains an important means of economic development, with oil and natural gas accounting for 57.5% of global primary energy consumption. While transportation safety records have steadily increased, some spills do occur. In the summers of 2018 and 2019, controlled and contained spills of diluted bitumen (dilbit) were introduced into enclosures installed in Lake 260 at the IISD-Experimental Lakes Area (IISD-ELA) in Northwestern Ontario. These additions were performed to examine the fate and behaviour of oil in freshwater systems and to compare the efficacy of selected cleanup techniques as part of the Boreal-lake Oil Release Experiment by Additions to Limnocoals (BOREAL) and Freshwater Oil-Spill Remediation Study (FOReSt) studies, respectively. These projects included rigorous containment and contingency measures. The efficacy of those measures and the potential for impacts to early development of lake trout arising from exposure to residual oil was examined by comparing morphology and oxidative stress in early life stages of lake trout obtained from the lake where oil studies were conducted and unimpacted reference lakes. Lake trout are a species known to be sensitive to oil constituents, most notably polycyclic aromatic compounds (PACs). Eggs and milt were collected from fish in the four study lakes (n = 3-7). After transport, dry fertilizations were performed, and the fish were reared in Heath trays until swim-up. A subsample of fry from each lake (n = 6-30 per maternal clutch) were evaluated for spinal, craniofacial, edema and finfold deformities, with no increase in deformity rates being observed in the study lake, compared to the unimpacted reference lakes. Fertilization success, egg mortality and hatching success were also similar among lakes (p > 0.05). While no phenotypic changes were observed in fish from the lake where oil studies were conducted, we also evaluated genetic and biochemical markers of PAC exposure. Cytochrome p4501A (CYP1A), a common biomarker of PAC exposure in fish, and the ratio of oxidized and reduced glutathione, an indicator of oxidative stress, were measured in a subsample of whole swim-up fry and levels were found to be similar between the study lakes (p > 0.05). Results from this study will be used to evaluate the efficacy of containment and contingency measures and the trajectory of recovery in oil spill impacted freshwaters.

3.05.P-Tu130 Simultaneous Determination of Allium Compounds (Propyl Propane Thiosulfonate and Thiosulfinate) in Animal Feed Using Uplc-Ms/Ms

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Propyl-propane-thiosulfonate (PTSO) and Propyl-propane-thiosulfinate (PTS) are organosulfur compounds present as secondary metabolites in many plants of the genus *Allium*. They are used to supplement the diet of livestock because of their beneficial effects on palatability, antimicrobial and antimethanogenic activities in order to reduce the greenhouse gas levels in the environment, and to improve the livestock efficiency. Furthermore, since antibiotics used in livestock as growth promoters were forbidden by European legislation due to the unwanted residues they generated, additives that come from a natural origin are considered the best alternative. However, to guarantee the safety of these sustainable additives, a correct analytical validation is necessary. For this reason, the objective of this study was to optimize the extraction parameters for the analysis of PTSO and PTS in feed matrices by performing a solid-liquid extraction and quantification by Ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Optimization was performed using the Response Surface Methodology on a Box-Behnken experimental design, optimizing the following parameters: solvent:sample ratios and evaporation temperature set for the rotary evaporator. The method was validated for 3 concentration levels for both PTSO (100, 500, 1000 ng g⁻¹) and PTS (500, 1150, 2300 ng g⁻¹). The highest recoveries of PTSO and PTS were obtained using 12.5 mL of 100% acetonitrile, stirring for 15 min, and an evaporation temperature of 20°C. The validated method was further applied to detect and quantify these compounds in different feed matrices (ruminants, broilers, pig, lactating pig, hens and fish). In conclusion, this is the first study to simultaneously analyse PTSO and PTS at low concentrations, employing a sensitive technique such as UPLC-MS/MS. This suggests the possible application of the proposed method in the industry and monitoring programs of feed additives, to ensure safety and efficacy. Acknowledgements: Spanish Ministerio de Ciencia, Innovación y Universidades (RTC-2017-6199-2) and

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3.05.P-Tu131 Investigation on Selection Efficient Representative Matrices for Multi-Residue Analysis of Pesticides in Agricultural Products

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Recently, simple and efficient multiresidue pesticides analytical methods using QuCHERS and mass spectrometry have been developed in many countries for the safety management of pesticides residues in food. However, the accurate quantification of pesticide residues requires the preparation of the same matrix for each sample of agricultural products therefore there is difficulty in using a mass spectrometer in such analysis. In addition, nowadays, matrix matched analysis is important in pesticides analysis and essential technique in quantitative analysis of pesticides residues using a mass spectrometer. However in the case of simultaneous multi-residue analysis, various agriculture samples should be prepared, but it is almost impossible to prepare a blank matrix for all agricultural sample matrices. Therefore, this study is proposed to investigate the possibility of selecting a representative matrix that can be used in the multi residue analysis of 460 pesticides active ingredients in various agricultural products. Fifty highly consumed types of agricultural products in South Korea will be selected and analyzed for the residues of 460 pesticides ingredients included in the Korean Food Code and to calculate the matrix effects (%) for the various agricultural products and pesticides components as well. The Organization for Economic Cooperation and Development (OECD) and Korean Ministry of Food and Drug Safety (MFDS) guidelines will be followed to secure the validation of the analytical method, linearity, accuracy, repeatability, instrument limit of detection (I-LOD), and limit of quantification (LOQ). The expected findings of the proposed work could explore representative matrices that can be used to analyze 460 pesticide components in various types of agricultural products, and minimize the percentage of matrix effect. The expected output of this investigation could improve the applicability and efficiency of multi component residue analysis of pesticides in food and significantly contribute to the safety management of pesticide residues in Korea and worldwide.

3.05.P-Tu132 Determination of Ethylene Oxide and 2-Chloroethanol in Food and Food Additives

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Within the EU ethylene oxide (EtO) and its degradation product 2-chloroethanol (2-CE) have been banned since 1991. However, in many parts of the world it is still regularly used as a fumigant to kill bacteria and fungus on food and food additives, known as cold gas sterilization. The ban in the EU is based on EtO's toxicity and reactivity, causing mutagenic effects. Therefore, the EU has defined that there is no safe level of exposure for consumers and implemented Regulation 396/2005, stating that EtO must be determined above an LOQ of 0.05 mg/kg. This has led to numerous recalls of foods across the EU and the need to determine EtO from food and food additives. EtO poses several challenges during analysis, leading to complex sample preparation steps required to analyse EtO and 2-CE reliably. These challenges include EtO's high volatility and its high reactivity causing a transformation of EtO to 2-CE very rapidly. Other methods circumvent this challenge by transforming EtO into its degradation product. New advancements in headspace sampling technology by linking it to a backflushed focusing trap have made it possible to extract both compounds from the food matrix, by extracting the food sample multiple, sequential times. This multi-step enrichment headspace-trap (MSE-HS-trap) technology allows the headspace of the sample to re-equilibrate, while other samples can be extracted. Here a new sample extraction technique is presented for the analysis of EtO and 2-CE from foods using MSE-HS-trap, where three enrichment steps were necessary to reach the required LOQs specified by the EU regulation. Unlike other methods, EtO and 2-CE are extracted, preconcentrated (on a sub-ambient, cryogen-free focusing trap) and analysed at the same time, with no conversion step required to transform either EtO into 2-CE or vice versa. The calibration range for each of the compounds ranged from 0.013–0.25 mg/kg, which bridges the 0.05 mg/kg cut-off for EtO. With this methodology sesame seeds were obtained from a rejected batch bound for Germany. EtO, within the batch by itself, was found to be just above the limit of 0.05 mg/kg (0.057 mg/kg) and the concentration of 2-CE was found to exceed the limit by far (8.760 mg/kg EtO equivalent). This methodology offers a fast and low manual labour approach to determining EtO and its corresponding degradation product from sesame seeds. Headspace-trap with MSE is well suited and can be adapted to many kinds of sample matrices.

3.05.P-Tu133 Method Development for the Analysis of C₆₀ and C₇₀ Fullerenes and Their Derivatives in Aerosol and Settled Dust

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Even if their effects on human health and environment are still debated, as their industrial production reaches around tens of tons per year, fullerenes and their derivatives should be considered as emerging contaminants (Astefanei et al., 2014; Carboni et al., 2016). For further toxicological risk assessment studies, more analytical data reporting the occurrence of these novel pollutants in various environments are critically missing. To our knowledge, only three previous works assessed their occurrence in aerosols, two of them focusing only on the sole C₆₀ fullerene (Benn et al., 2012; Encinas and Gómez-de-Balugera, 2018; Sanchís et al., 2012). In our work, we developed a single method for the simultaneous analysis of C₆₀ and C₇₀ together with three fullerene derivatives, frequently used for industrial applications (Sanchís et al., 2012). The very high hydrophobicity of fullerenes requires special analytical conditions. Above all, we report the necessity of using toluene both as extracting solvent and as liquid

chromatography (HPLC) eluent as it seems to be the best solvent for fullerenes. Therefore, we applied Pressurized Liquid Extraction (PLE) with a Toluene / Acetonitrile (1:1, v:v) mixture to extract quantitatively our analytes. Extraction yields measured on a real particulate matter (PM) matrix were found to be from 67% to 104% for our five target compounds. HPLC was successfully applied to separate our fullerenes on a C₁₈ stationary phase (150 x 3 mm, 3 µm) in 10 minutes. In this work, we extensively compared the performance of the three most common atmospheric pressure ionization techniques: ESI, APCI and APPI, in both positive and negative modes. The electron capture electrospray negative ionization was found to be less robust and sensitive than APCI and APPI. Negative APPI was finally selected as the optimal ionization method for most compounds, except NMFP which showed a higher signal in positive mode. As fullerenes show very little fragmentation, the pseudo-MRM mode was implemented for fullerenes monitoring, which means that the selected m/z transitions did not exhibit any mass loss from parent ion to daughter ion. LC-APPI-MS/MS instrumental quantification limits (LOQs) were from 10 to 50 pg injected for the five fullerenes, enabling to reach method LOQs down to 1 µg/g in settled dust and down to 0.02 ng/m³ in airborne PM. Thanks to these low LOQs, the developed ASE-LC-MS/MS method was successfully applied to aerosol and settled dust samples collected in indoor urban environments in the area of Strasbourg, France.

3.05.P-Tu134 Increasing the Sensitivity for 1,4-Dioxane Analysis in Drinking WATER

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In past water investigations at solvent release sites, 1,4-dioxane was typically not included as a target analyte because it was not detectable at low concentrations in a standard laboratory scan for volatile organic compounds. However, 1,4-dioxane has emerged as a likely human carcinogenic compound, and as it is highly soluble and does not readily biodegrade it tends to accumulate in the aquatic environment. This has led to a large spread in 1,4-dioxane contamination sites. Further, attitudes towards 1,4-dioxane have changed in recent years. This is reflected in the recent decrease of regulatory detection limits, in some cases to 25 ng/L (Bayerischer Landtag 17/16517). Reliable low-level detection of 1,4-dioxane is challenging because of its high solubility, which makes it particularly difficult to extract from water. A combination of solid-phase microextraction (SPME Arrow) with further preconcentration on a sub-ambient trap allows for multi-step enrichment (MSE), wherein analytes from multiple SPME Arrow extractions from a single sampling vial are concentrated prior to trap desorption. With this technique, 1,4-dioxane is removed from the headspace of the sample with each extraction, and time is then allowed for a new equilibrium to establish prior to the following extraction, forming a semi-dynamic headspace approach, dramatically improving extraction efficiency overall. SPME Arrow-trap with MSE thus allows analysis of 1,4-dioxane at trace levels far surpassing what is possible with traditional SPME Arrow while maintaining peak shape, excellent linearity, low MDLs and highly reproducible results. During the development of the method it was determined that three enrichment steps of MSE-SPME Arrow-trap were necessary to obtain results with the required sensitivity, while allowing the cycle time of the methodology to remain below one hour. Furthermore, by encompassing the sub-ambient trap with SPME Arrow, this method can perform re-collection and archiving of SPME Arrow extracts for later re-analysis if required on thermal desorption tubes. Here we demonstrate the fully automated sampling and detection of the 1,4-dioxane at ng/L levels in water using a multi-phase (DVB/CWR/PDMS) Arrow for MSE-SPME Arrow-trap.

3.05.P-Tu135 Chromatographic Determination of Selected Carbamate Pesticides in Soil Samples: Degradation and Bioavailability

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Pesticides are generally applied to agricultural crops for protection but their residues present a potential risk to non-target organisms, affecting aquatic ecosystems and posing risks to human health. Organophosphates and carbamates impair nerve transmission in insects and poses even higher human health risks if left undetected. Recent advances have shown that these pesticides can be extracted and pre-concentrated from aqueous samples by liquid-phase micro-extraction (LPME), solid phase extraction (SPE) and headspace solid phase micro extraction (HS-SPME), while supercritical fluid extraction (SFE) and pressurised fluid extraction (PFE) can be employed for sediments. This study has shown that improved extraction allows for sensitive detection of carbamates in soil and fruit samples. The study involves the testing of a rapid, reliable and low-solvent extraction procedure for selected carbamate pesticides of carbaryl, carbofuran, and methomyl from soil and fruit samples. It involves an investigation to determine how much of the applied carbamates are found in the fruit, compared to the soil in the same geographical location. This study is currently investigating the chemical behaviour of the carbamates in soil under environmental conditions and aims to examine if a sonicated SPE procedure promotes the release of the carbamates in the soil, before samples are subjected to a GC-MS/MS or LC-MS analysis procedure.

3.05.P-Tu136 Analysis of OTA and OTB in Urine Employing Lc-Q TOF MS and Exposure Assessment Study

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Traditional evaluation exposure to mycotoxins is often carried out combining chemicals analysis in foodstuffs with data of food consumption. This indirect approach presents some disadvantages such as the lack of information related to individual exposure situation, toxicokinetics and mycotoxins bioavailability and the difficult to obtain accurate data on food consumption. Human biomonitoring constitutes a suitable alternative to assess toxins exposure overcoming the limitations of the indirect approach (1,2). Mycotoxins are secondary metabolites produced naturally by filamentous fungi that produce a wide range of adverse health effects. *Aspergillus* and *Penicillium* genera are responsible of Ochratoxin A (OTA) production (3). OTA constitutes a toxic compound with relatively rapid absorption and slow elimination, being the kidney and liver, the principal organs involved in its

biotransformation. The OTA metabolite, Ochratoxin B (OTB) is considered less toxic than the parent compound (4). The aim of the present work was to evaluate the presence of OTA and its metabolite OTB in human urine samples and to estimate the potential risk of exposure. For this purpose, 56 urine samples were extracted by QuEChERS method and determined by LC-ESI-QTOF-MS. The samples were acquired from 24 males and 32 females in a wide age group. The results revealed that OTA was not detected in any of the analyzed samples, while OTB was observed in 9% of samples with concentrations ranging from < LOQ and 38.88 µg/L, and mean of positives of 18.17 µg/L. Comparing between genders, the mean amount detected in male samples (38.88 µg/L) was higher than those detected in females (12.99 µg/L). The risk assessment study revealed that PDIs calculated for OTB exceeded the TWI established for OTA. Moreover, the margin of exposure (MoE) obtained employing the BMDL10 established for OTA also revealed a potential health risk with MoE values

3.05.P-Tu137 Identification and Characterization of Non-Petroleum Oils in Support of Emergency Response and Ecosystem Assessment

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With the advent of containerized trade and the streamlining of supply chains between container ports globally, oceans and waterways have becoming increasingly important transport routes for cargo ships. The volume of seaborne trade has nearly doubled over the past 20 years, reaching 11 billion tons loaded in 2019. The upward trend continues with a current estimation of 80 percent of good transported by ships. Even as non-petroleum oils are growing component of global bulk transport, there are minimal data on the chemical and physical properties of these oils. The increase in global transport as well as recent spill incidents involving non-petroleum products highlight the need for advancements in extraction methods and analytical identification of biogenic and non-petroleum oils. In 2020, the Alaska Sea Grant Marine Advisor in Nome reported a mystery substance on the beach and coating a number of seabirds in the village of Savoonga. Initial responses suggested the substance was likely a non-petroleum oil such as a vegetable oil, fish oil, spent cooking oil, or type of rendered fat, released, then solidified in the cold water. Samples were analyzed via standard GC/MS methods for semi-volatile compounds and the results determined the constituents in the unknown matrix were of non-petroleum origin. In addition to qualitative differences, including patterns of alkanes and PAHs unlike from typical petrogenic fuels and oils, the unknown substance contained undetectable concentrations of the 16 EPA Priority PAHs and no high molecular weight aromatic compounds. As a result, it was recognized that additional analytical tools were necessary and research has begun to develop an effective extraction method for the identification and characterization of non-petroleum oils via GC/MS. In addition to the characterizations of chemical properties of non-petroleum oils, biogenic oils will be analyzed for bulk physical properties such as density, viscosity, emulsification, and more. The current research attempts to characterize chemical and physical profiles for high priority, novel non-petroleum oils, ultimately building a reference library to aid in source identification of spilled substances in nearshore and coastal environments.

3.05.P-Tu138 QualiMon LaMa - Live Quality Monitoring of Non-Targeted Analysis Through Utilization of Landmarks

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Technological advancements have taken mass spectrometry into the omics realm, enabling non-targeted analysis of thousands of compounds within a single sample. Like all new technologies, high resolution mass spectrometry faces several obstacles on the road to reach its full potential: While high resolution and sensitivity are what make these techniques attractive for nontarget analysis, it also makes the instruments susceptible to drift-related disturbances that are detrimental for data quality. Unfortunately, these disturbances are generally not detected during instrument analysis and inadequate instrument performance or data quality is often discovered during data processing of individual batches or full experiments, potentially comprising thousands of samples. This can lead to severe consequences, such as precious sample material being wasted and increases in required instrument-time and burdens for technicians. To address this problem, we have developed “QualiMon – LaMa”, a near-real-time Quality Monitoring software. At the core, the software monitors LandMarks (LaMas), i.e. unique, easily distinguishable features that are present in virtually all samples and/or quality control standards of a particular matrix. LaMa characteristics (such as rt, m/z, intensity, s/n) are automatically extracted on an injection-by-injection basis into a local database structure. QualiMon then detects any significant deviations from the previous injections as well as violations of instrument-specific cut-off limits. Transgressing samples activate real-time warnings through web services, so that instrument technicians can take appropriate measures immediately. LaMas also have the benefit of providing a framework for improved retention time alignment between samples, batches and can be utilized to improve matching to in-house databases for identification purposes. QualiMon is developed in open source (R and SQLite) and will be available for free under license. In conclusion, QualiMon offers assessment of instrument performance and data quality mid-sequence and the possibility to minimize waste of resources through live monitoring of non-targeted data.

3.05.P-Tu139 Are Natural Deep Eutectic Solvents Always a GREEN Option? A Bioassay-Based Study

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The traditional use of organic solvents in various branches of industry is being rethought as these compounds (e.g., dimethylsulfoxide (DMSO)) very often display high volatility, toxicity and lipophilicity (related to the ability to interact with biological membranes). More recently, developments in the field of Green Chemistry are focusing on the design of more sustainable and cost-effective solvent alternatives like Ionic Liquids (ILs), bio-based solvents and natural deep eutectic solvents

(NADESs). The present study aimed to perform an ecotoxicological screening of 15 NADESs compounds using an extensive set of marine and freshwater bioassays, which aimed at different endpoints: immobilization of the freshwater crustacean *Daphnia magna*, inhibition of growth of the freshwater alga *Raphidocelis subcapitata*, and of the marine alga *Phaeodactylum tricoratum*, alteration in larval development on the brackish water serpulid *Ficopomatus enigmaticus* and the inhibition of bioluminescence in the marine bacteria *Aliivibrio fischeri*. The latter was performed using both freshwater and marine water protocol. What emerged was a clear absence of effect of all samples against *D. magna*. Regarding *F. enigmaticus*, highest effects were observed with 2 choline acetate- based compounds, in particular the one with imidazole and the one with citric acid. *A. fischeri* showed, in general, differences between the marine and the freshwater assay on same compounds. Specifically, for marine assay, bacteria showed a low degree of bioluminescence inhibition, while for freshwater assay a stimulation of bioluminescence was observed. Both marine and freshwater algae showed a certain degree of biostimulation. *P. tricoratum* growth was stimulated mainly by proline-based compounds, while *R. subcapitata* growth was stimulated by almost all tested compounds. Since biostimulation of algal growth is actually considered as an effect, samples which exhibited an increase of cellular concentration over 40% respect to control, were retested. The new assays were performed in the range 1-100 mg/L (8 dilutions) in order to find statistically significant NOEC and LOEC. Despite no ecotoxicological parameters, such as EC20/50, were calculable, biostimulation endpoints may underline a potential risk linked to substances considered “green”, in terms of environmental degradation.

3.05.P-Tu140 A Cost- and Time-Effective Screening Method for Determining Biodegradability Using Non-Invasive Optical Oxygen Sensors

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Improved biodegradability of chemicals in the environment is a key element for all actors in the supply chain as well as for the assessment of substances in existing and evolving regulatory frameworks. Especially, but not exclusively, for polymers it is and continues to be increasingly important to avoid persistent residues that could harm the environment and human health.

Hence, there is a world-wide research effort to increase the biodegradability of organic substances and polymers. Already, during the development of new polymers sustainability and sustainable-by-design plays a crucial role.

However, the state-of-the-art process to assess the biodegradability of substances is time consuming and expensive. With the presented screening method established by Noack and Clariant, it is possible to obtain an initial indication of the percentage biodegradability of several substances and polymers at a time. The turnover time is significantly reduced, by reducing the time for preparation and evaluation and the costs are decreased compared to the standard process.

For this purpose, up to ten different substances can be tested within a variable duration under flexible conditions in the innovative measuring system. The biodegradation is calculated via the oxygen concentration in the test vessels, which is determined via non-invasive optical oxygen sensors. This allows any form of substance or polymer to be tested, regardless of whether it is water-soluble or not.

The functionality of the test system has already been demonstrated in tests with various reference materials with known degradation behavior (fast, medium and slow biodegradation and polymeric alcohol ethoxylates) as well as with promising candidates from research and development activities to improve the biodegradability of polymers from different classes.

In summary, the established screening system offers the opportunity to assist the sustainability-focused innovation-pipeline by helping to decide about promising candidates for better biodegradable substances and polymers with reduced time-demand and costs.

3.06 Air pollution: exposure and effects on human and environmental health

3.06.T-01 Why Is the Air Toxic? Virtual Effects-Directed Analysis (vEDA) to Identify Human Health Hazards in Fine Particulate Matter (PM_{2.5})

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Air pollution causes major threats to human health across the globe. The air quality in South Korea is an increasing concern and is highly affected by continental outflows of neighboring countries. Depending on the source of the air, weather, and season, the composition of fine particulate matter (PM_{2.5}) is highly variable. Therefore, the present study uses a *virtual effects-directed analysis (vEDA)* approach to identify toxic chemicals. In the vEDA approach, each sample does not need to be fractionated. Rather, many samples are tested directly with bioassays and each sample is also comprehensively characterized with non-targeted mass spectrometry analysis. Through this ‘big data’ approach, the study aims to identify chemical features that correlate with toxicity in relevant human cells. PM_{2.5} samples were collected continuously from the Korean Climate Observatory at Gosan (33.29° N, 126.16° E), located in the Yellow Sea on the South Korean island of Jeju. Collection period of PM_{2.5} samples was over 17 months and the water-soluble component of the filters was extracted. A battery of bioassays and chemical analyses were carried out on the extracts. The samples were tested in cellular cytotoxicity and reactive oxygen species (ROS) assays (in primary human nasal epithelial and lung fibroblast cells) and an cellular assay (the dithiothreitol, or DTT, assay). Comprehensive screening of the water-soluble extracts was performed by HPLC-HRMS (in data independent acquisition mode) while trace metal analysis was carried out by ICP-MS. Two days back-trajectories of the air reaching the sampling station was modelled every hour, and aerosol scattering coefficients as well as particle sizing were measured during the sampling campaign. The bioassays showed

a high variability of the toxicity depending on the sampling days justifying the vEDA approach. The non-targeted analysis of PM_{2.5} extracts detected thousands of features. The deconvoluted MS2 spectra were compared against high-resolution spectral databases. The unmatched features were processed with high-throughput software workflows to predict their molecular formulas, and molecular networking was then applied to group molecules which have similar structures, based on spectral similarities. Together with the atmospheric parameters, the list of toxic candidates is being refined. This project is a unique opportunity to understand the challenges we are facing in terms of atmospheric exposure to toxic compounds.

3.06.T-02 Nontarget Mass-Spectrometry and In-Silico Molecular Characterization of Air Pollution From the Indian Subcontinent

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Airborne fine particulate-matter (PM_{2.5}) is a major component of air pollution that contains unresolved complex mixtures of organic compounds. A deep molecular characterization of PM_{2.5} would contribute to improved understanding of air pollution impacts on global climate and human health. Here, we collected PM_{2.5} for 3 months at a remote receptor site in the Maldives that regularly intercepts polluted air outflows of the Indian subcontinent, and occasional pristine air from the Southern Indian Ocean. To achieve a broad molecular characterization of organic compounds, each PM_{2.5} sample ($n = 40$) was extracted with a range of solvents and analyzed by combining gas- and liquid chromatography high-resolution mass-spectrometry, i.e. (GC)- and (LC)-HRMS. A wide range of known anthropogenic contaminants were confirmed, including legacy persistent organic pollutants, polycyclic aromatic hydrocarbons (PAHs), plasticizers, flame retardants, pharmaceuticals, pesticides and associated transformation intermediates, some of which were detected in air for the first time. Unprecedented complexity was observed by nontarget analysis (>60,000 features). However, only a minor proportion of features (0.5%) could be annotated with spectral databases (318 compounds) or identified with authentic standards (53 compounds at Level 1, and 36 compounds as closely related isomers) - thus leaving vast 'molecular dark matter' structurally uncharacterized. An integrated workflow that leveraged on molecular networking and *in-silico* fragmentation predictions allowed us to reach a structural-level characterization for over 10,000 molecules, of which over 1,000 were hallmarks distinguishing polluted air of the Indian subcontinent from pristine air of the Indian Ocean. Clean air from the Indian Ocean primarily contained simple organooxygen molecules (O_x class; x=1-3) while polluted air was dominated by organodinitrogen (i.e. N₂, N₂O_x class), organosulfur and highly-oxygenated molecules (O₄-O₇ class). Overall, molecules associated with polluted regions occupied a broader and highly oxidized chemical space with physicochemical properties of high relevance to human toxicity and regional climate. Altogether, these results highlight how nontarget analyses and *in-silico* structure predictions can be implemented as advanced tools to explore deeper molecular-level insights and hypotheses on the health and climate impacts of complex organic compound mixtures in airborne PM.

3.06.T-03 New Approach Methodologies (NAMs) and Chemometrics to Study the Health Effects of Air Chemical Mixtures on Lung Cells

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Airborne particulate matter (PM) pollution has raised great concern since its exposure has been associated with adverse respiratory outcomes, such as exacerbations of asthma and chronic obstructive pulmonary disease, pulmonary fibrosis and lung cancer. However, the causality between specific chemical exposures and their effects on lungs health and their mechanism of action remain mostly unknown. To avoid the use of animals, a variety of approaches called New Approach Methodologies (NAMs) have been developed and progressively introduced to improve understanding of toxic effects of chemicals. In this work, we propose the use of NAMs, i.e. 3D cell cultures, HTS methods (viability assays, ELISA) and lipidomics to assess the biological effects of chemical extracts from environmental air samples. In addition, we apply chemometrics methods (PCA, MCR-ALS) to find relationships between the chemical composition data of air samples and the biological effects on cell cultures. Filter samples from an air quality monitoring station of Barcelona (collected from June to December 2019) were characterized using ICP-MS and GC-MS, and source apportionment was carried out using Positive Matrix Factorization. The results about the biological effects showed that sample extracts caused important changes on the release of pro-inflammatory cytokine IL-8 and on the lipidomic profile of 3D lung cell cultures. Different patterns of lipid changes and distribution along time were observed in the MCR-ALS analysis of lipidomics data from PM1 and PM10 treated cells. Data fusion of chemical analysis of PM1 and PM10 filters, and the biological effects data was performed to find a relationship between specific compounds or sources of pollution and changes in lipids and phenotype. Correlation maps and MCR-ALS analysis were applied to this fused data showing specific lipid patterns associated to pollution sources. Overall, the use of three dimensional lung cell cultures, HTS assays, omics and chemometrics in this work provided information about the biological mechanisms induced by PM1 and PM10 air pollution samples. The application of MCR-ALS on the chemical and biological fused data provided insight about the chemical pollutants and sources of pollution that contribute to the changes observed in lung cells. Additional data is being generated to produce more robust models that enable the prediction of biological outcomes using the chemical composition of samples.

3.06.T-04 Influence of Temperature Inversions on Organic Aerosols and Human Exposure: Measurement by Using Meteorological Balloons

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Outdoor air pollution constitutes a global threat to ecosystems and human health. In 2016, around 4.2 million premature deaths worldwide were attributed to this cause and, still in 2019, 99% of the world population was breathing air which does not fulfil the WHO air quality guidelines levels. The highest human exposure to airborne pollutants occurs in cities which is usually more related to atmospheric conditions than to changes in emission sources. Atmospheric temperature inversion conditions often involve precautionary health warnings as they increase the concentration of atmospheric pollutants. Restricted dispersion of organic compounds and photooxidation in the upper air layers lead to qualitative and quantitative changes of aerosols with formation of secondary organic aerosols (SOA), involving processes that are still largely unknown. Understanding the main changes in composition of these compounds at molecular level will help to define the best strategies to deal with the improvement of air quality and reduction of health risks. The present study is devoted to the analysis of the vertical distribution of particle-associated organic pollutants and Black Carbon (BC), which was performed with the use of captive balloons equipped with particulate matter (PM) samplers and BC monitors. The qualitative and quantitative vertical distribution of organic pollutants was studied under standard environmental lapse rates and temperature inversion conditions. The organic pollutants present under several types of temperature inversions in urban and rural environments in different seasons were studied. One of these environments was located in the semiurban area of Osona (Catalonia) where the highest winter benzo[a]pyrene concentrations have been observed among those recorded in the Iberian Peninsula. The other site is a rural background site surrounded by fruit fields. The temperature inversions in the plain systems and associated atmospheric transport of organic pollutants, prior and after photooxidation, have been studied. The particle-associated pollutants involved polycyclic aromatic hydrocarbons (PAHs), anhydrosaccharides, hopanes, phthalates, and SOA compounds. Some of them were used as tracers to identify the contribution of emission sources and secondary formation to PM. The chemical analysis was performed with a high-resolution Q Exactive GC Orbitrap GC-MS/MS (GC-Orbitrap-MS).

3.06.T-05 Ecotoxicity of Atmospheric Particles' Deposition on Marine Organisms

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Currently, air pollution is a growing problem globally, mostly due to industrialization and urbanization. Anthropogenic activities can contribute to the emission of several pollutants into the atmosphere, including particulate matter (PM). Despite the few existing studies on literature, it can be predicted that the deposition of such atmospheric PM on the water bodies can affect marine biota performance. The magnitude of these effects depends on the atmospheric concentration and composition of PM and the wet/dry status of this PM in the deposition process. Using the NIST SRM® 1648a material as a surrogate for urban PM, this work considered the deposition of PM equivalent to one year of oceanic deposition (11.4 mg NIST/L) in an attempt to understand the toxicological effects on different marine organisms. Producers (microalgae and diatoms) and primary/secondary consumers (rotifers and polychaete) were used aiming to partially cover the current knowledge gaps on this topic. Thus, growth inhibition of primary producers and survival of rotifers and polychaetes were assessed. In addition, biochemical changes in polychaetes were measured, regarding oxidative stress (LPO-lipid peroxidation), antioxidant enzymes (CAT-catalase, GPx-glutathione peroxidase, GR-glutathione reductase), detoxification enzymes (GST-glutathione-S-transferases), neurotransmission impairment enzyme (AChE-acetylcholinesterase). Significant differences were observed between the control organisms (artificial saltwater with salinity 35) and exposed organisms in primary producers. Effects differed between diatoms and golden-brown microalga *I. galbana*, with diatoms being stimulated to grow while microalgae being inhibited. One possible explanation was hypothesized regarding the presence of silicon in NIST particles ($12.8 \pm 0.4\%$), which could promote the growth of diatoms since the chemical and structural composition of their frustules is highly dependent on this element; on the other hand, the presence of contaminants in the NIST particles may justify the growth inhibition on *I. galbana*. Furthermore, no acute effects were observed in the tested animals, however significant oxidative stress (LPO) and glutathione peroxidase activity (GPx) impairment were detected. The results suggest that PM does indeed cause effects on marine biota.

3.06 Air pollution: exposure and effects on human and environmental health (Poster)

3.06.P-Tu141 Source Apportionment and Toxicity of PM in Urban, Sub-Urban and Rural Atmospheres in Catalonia

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Exposure to air pollution is a global threat to human health and ecosystems. Effective action to reduce air pollution requires a good understanding of sources, transport and transformation mechanisms and the effects of these atmospheric pollutants on humans and ecosystems. The contributions of biomass emissions to PM₁₀ and BaP, as well as the formation of secondary organic aerosols (SOA) and their influence on PM and their toxicity are still pending of clarification. PM₁₀, NO₂, O₃ and BaP and several molecular organic tracer compounds (e.g. levoglucosan, hopanes, SOA) were evaluated in three geographically different sites in Catalonia (Spain) that often exceed the limit values in the past decade. The organic compounds were analyzed by GC-MS and toxicity was evaluated by the pulmonary lung cells A549 assay. The PM samples were collected in an urban traffic site, a sub-

urban background site, and a rural background site, from summer to winter 2019. Source apportionment of the organic aerosol confirmed the traffic related air pollution in the urban site, and showed that biomass burning and secondary organic aerosols impact the air quality in the sub-urban and rural background sites, with substantial differences between summer and winter. Toxicity tests of the PM extracts with lung cells show higher toxicity (Inhibition of Cell growth) in wintertime samples in the sub-urban and rural sites, compared to the urban site and summertime sample with high biogenic SOA contributions. The inhibition of cell growth (IC50) was observed at equivalent air volumes between 4 and 30 m³, which were relevant volumes for daily respiratory volumes in humans. IC50 correlated best with biomass burning indicators, PAH, and quinones. These results that there is a need for different mitigation strategies for urban and rural sites in order to improve the air quality and reduce health effects. In urban areas traffic emissions are still the dominant factor influencing urban air quality and may directly be responsible for part of the SOA and O₃ levels in suburban and rural areas, while these later areas cope with putative air quality from local biomass burning emissions, essentially in the colder period of the year. *Reference* - Clara Jaén, et al. 2021, doi: 10.3390/atmos12060744 *Acknowledgement* - Financial support for this study was provided by the research projects from the Plan Nacional de I+D of the Spanish Ministry of Science and Innovation CUANTOX (CTM2015-71832-P) and INTEMPOL (PGC2018-102288-B-I00).

3.06.P-Tu142 Cytotoxicity of Human Lung Cells (A549) After Exposure to a Gaseous Bio-Hybrid Fuel Blend at Air-Liquid Interface (ALI)

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Emissions from the transport sector contribute to air pollution and lead to exposure of humans to a variety of gaseous chemicals. Recently, several studies have shown that gaseous chemicals induce adverse effects, e.g., programmed cell death, inflammatory responses, or DNA damage on cell level. However, most studies focussed only on single substance exposure whereas mixtures of several chemicals are present in the environment. In addition, the investigated exposure pathway, e.g., using cell suspensions might lack relevance to human lung physiology. Therefore, we exposed human lung cells to a gaseous bio-hybrid fuel blend mixture at air-liquid interface (ALI) conditions. Briefly, A549 cells were cultivated on microporous membranes supplied with media from the basal side. Then A549 cells were exposed apically to a 20 mL/min flow of undiluted or diluted gaseous fuel blend (components: methanol, ethanol, methyl acetate, ethyl acetate, 3-methyl-2-butanone and n-pentane) for 1 h at ALI in a humidified air at 37 °C. The investigated fuel blend was chosen based on previous work from Ackermann *et al.* 2021. After exposure, cells were post-incubated for 24 hrs and cell viability/toxicity endpoints were investigated. Microscopic investigation revealed a damaged cell layer of exposed cells compared to controls (qualitative). Furthermore, our results indicated a slightly lower cell viability of exposed cells for both undiluted and diluted exposures compared to controls (quantitative). Our experiments resembled an exposure scenario, e.g., to simulate inhalation of highly concentrated fuel vapours at the gas station or in low-ventilated areas. For future research, other factors such as the oxidation products of the chemicals and the involvement of other gaseous compounds in the tested mixture could contribute to a more realistic exposure scenario. This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – Cluster of Excellence 2186 „The Fuel Science Center” – ID: 390919832.

3.06.P-Tu143 Relationship Between Airborne Pollen Concentrations and Air Pollutant Levels in a LOW Polluted City

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Scientific studies on health impacts caused by air components focus on the independent effects of the levels of atmospheric pollutants and aerobiological particles. In contrast, there are less studies that relate both types of data. The objective of this study is exploring statistical relationships in Badajoz (SW, Spain) among airborne pollen concentrations of different pollen types such as Quercus, Olea, Poaceae, and Pinus, and air pollutants as O₃, CO, NO, NO₂ and SO₂. The relationship between atmospheric pollutants and pollen grains was studied using Spearman's correlation (r) (significance level of 95%), from 2010 to 2019. The levels of the different pollen types were obtained from aerobiological sampling using a volumetric sampler. The levels of atmospheric pollutants were acquired from the city's air quality monitoring unit. The correlations results vary according to the pollen type and pollutant. The correlations of O₃ with the different pollen types are higher than reported data [1]. Poaceae presents the maximum value of r for O₃ (0.46); in cited study the correlation is between 0.10 and 0.24. However, NO₂ presents similar negative correlations, between -0.10 and -0.20 [1]. Olea pollen type also presents negative values for CO, NO₂ and SO₂. On the contrary, O₃ and CO show positive values. For Pinus there is no agreement between the negative value obtained for NO₂ and the reported positive values [1,2]. Quercus shows correlations similar to published data, except for the relationship between Pinus and NO₂ levels. The interrelations between the levels of pollen and atmospheric pollutants present a high variability, and multiple factors such as meteorological factors may be involved, as well as synergies between pollutants or time lags.[1] P. Cariñanos, I. Foyo-Moreno, I. Alados, J.L. Guerrero-Rascado, S. Ruiz-Peñuela, G. Titos, A. Cazorla, L. Alados-Arboledas, C. Díaz de la Guardia, Bioaerosols in urban environments: Trends and interactions with pollutants and meteorological variables based on quasi-climatological series, *Journal of Environmental Management*. 282 (2021). <https://doi.org/10.1016/j.jenvman.2021.111963>. [2] A. Rahman, C. Luo, M.H.R. Khan, J. Ke, V. Thilakanayaka, S. Kumar, Influence of atmospheric PM_{2.5}, PM₁₀, O₃, CO, NO₂, SO₂, and meteorological factors on the concentration of airborne pollen in Guangzhou, China, *Atmospheric Environment*. 212 (2019) 290–304. <https://doi.org/10.1016/j.atmosenv.2019.05.049>.

3.06.P-Tu144 The Effects of Traffic Pollution Are Changes in Lichens Caused by Road Salt and Lead Stress

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Road transport is responsible for a large proportion of air pollution in urban ecosystems. With the increase in car use, road traffic pollution is considered to be a major threat to ecosystems. The impact of salinity and heavy metals as lead on physiological parameters of lichens was determined. Lichens *Evernia prunastri* and *Ramalina fastigiata* were exposed to different NaCl in combination of lead. The results of the experiment showed that both exposures affected the lichen membrane damage as indicated by increase in conductivity. The potential photosystem II efficiency (F_v/F_m) was not so susceptible to the impact of stressors compared to membrane integrity. The results show that combination of the traffic-related factors have negative effect on sensitive lichens. The results of the study supplemented the knowledge on the effects of transport-related pollution on organisms, such as salts and heavy metals, on lichens and provided a better understanding of the mechanisms of toxicity.

3.06.P-Tu145 Development of Extraction and Sample Preparation Methods for the Application of Metaproteomics to Bioaerosol

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The biological fraction of aerosols is of growing concern regarding its adverse health effects because of the airborne allergens and pathogens it contains [1,2]. Up to now, most analytical methods employed for the characterization of bioaerosols are either restricted to a particular type of biological source (such as optical microscopy for pollen grains and fungal spores), or they only give broad information on the whole bioaerosol without any selectivity (such as total protein content or molecular tracer quantification methods) [3,4]. Therefore, metaproteomics seems to be a very appropriate analytical tool for the characterization of bioaerosol [1,2]. Indeed, shotgun bottom-up analysis of the protein content of such environmental samples enables a large-scale characterization of their expressed phenotype regardless of their taxonomy, thus it can lead to the identification of the main protein sources in bioaerosols. However, the application of metaproteomics to bioaerosol samples is associated with two major analytical challenges: the data interpretation with bioanalytical tools and database searches, and the sample treatment, including extraction and further preparation steps. In this work, we describe the influence of various parameters such as: - the extraction time, - the number of extraction cycles, - the composition of the extraction solution in terms of detergents, pH buffers, etc... on the efficiency of ultrasound-assisted in-solution extraction of the proteinaceous content of bioaerosol samples. We also discuss the necessity of further sample preparation, including detergent removal, concentration and purification steps, which are often necessary but can also lead to protein loss. All the optimizations presented in this work were found to be critical for the enhancement of the detection of proteins present at very low levels in airborne particulate matter. **References** [1] F. Liu, S. Lai, K. Reinmuth-Selzle, J.F. Scheel, J. Fröhlich-Nowoisky, V.R. Després, T. Hoffmann, U. Pöschl, C.J. Kampf, *Anal Bioanal Chem.* 408 (2016) 6337–6348. [2] S. Piovesana, A.L. Capriotti, P. Foglia, C.M. Montone, G. La Barbera, R. Zenezini Chiozzi, A. Laganà, C. Cavaliere, *Proteomics.* 19 (2019) 1900152. [3] V.R. Després, J.A. Huffman, S.M. Burrows, C. Hoose, A.S. Safatov, G. Buryak, J. Fröhlich-Nowoisky, W. Elbert, M.O. Andreae, U. Pöschl, R. Jaenicke, *Tellus B: Chemical and Physical Meteorology.* 64 (2012) 15598. [4] S.J.R. Staton, A. Woodward, J.A. Castillo, K. Swing, M.A. Hayes, *Ecological Indicators.* 48 (2015) 389–395.

3.06.P-Tu146 Is There Any Potential Role of Essential and Toxic Trace Elements on COVID-19 Infection? An Observational Study in Catalonia (Spain)

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COVID-19 is the infection caused by the SARS-CoV-2 resulting in a massive outbreak in 2020. The most frequent symptoms include fever, cough, fatigue, sputum, and dyspnea. However, up to 20% of infected individuals undergo a severe disease, frequently leading to pneumonia, acute respiratory distress syndrome, organ dysfunction and death. Respiratory comorbidities, along with cardiometabolic diseases, cancer and long-term exposure to environmental pollutants are risk factors for the severity of COVID-19. Anyhow, the age is a key variable determining COVID-19 health outcomes due to the weakening of the immune system over the life course. Considering that both essential and toxic elements are responsible for immune responses, the present study was aimed at assessing their potential role on COVID-19 health outcomes. A retrospective observational study was performed with 214 individuals attending or admitted to the Hospital Sant Joan (Reus, Catalonia, Spain), which were classified into: i) individuals COVID-19+ with an asymptomatic infection course; ii) individuals suffering mild COVID-19; iii) individuals suffering severe COVID-19; iv) individuals COVID-19? (control). An *ad hoc* database with data on age, sex, toxic habits, comorbidities, blood test results, medication and clinical outcomes, along with the levels of As, Cd, Cr, Cu, Hg, Fe, Mg, Mn, Pb, Se, V and Zn in individual serum samples was created. Arsenic was < 0.1 µg/L in all individuals, while Hg showed a low detection rate (< 85%). None of the elements here assessed showed significant differences ($p > 0.05$) among the COVID-19 severity groups. However, the concentrations of Cd, Pb and V in infected individuals were significantly higher ($p < 0.05$) than the control group, while the levels of Zn in asymptomatic and mild COVID-19 were significantly lower ($p < 0.05$) than non-infected individuals. Although the occurrence of Zn in COVID-19 severe patients was higher than in COVID-19?, but with no significant differences ($p > 0.05$). Results revealed random correlations among analyzed elements. While Cd and Pb showed a significant ($p < 0.05$) positive correlation with C-Reactive Protein (CRP), glucose and aspartate transaminase (AST) in infected individuals. In conclusion, the present study indicates that exposure to Cd, Pb and V might weaken the immune system, and consequently, to increase vulnerability to develop COVID-19 in case of infection with SARS-CoV-2.

3.06.P-Tu147 Analytical Developments for the Assessment of 120 Pesticide Compounds in Blood, Plasma and Feathers of Birds

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More and more studies show the importance of biomonitoring as an evaluation tool to assess environmental contaminations by crop protection products. In this study we chose to analyze blood, plasma and feathers of *Perdix* to assess their potential contamination by those products. The group of pesticides is composed of a huge variety of compounds. Therefore, in this study, we optimized and validated methods to analyze around 120 compounds including herbicides, insecticides and fungicides. We compared various extraction methods found in the literature and we adapted the protocol of Goutner et al. (2011) for blood extraction and Hao et al. (2018) for the extraction of pesticides from plasma. To extract pesticides from feathers, we used a QuEChERS procedure following Humman-Guillemot et al. (2021). After extraction, samples were concentrated then injected simultaneously in LC-MS/MS and GC-MS/MS for quantification. Liquid chromatography was selected to separate non-volatile or thermolabile compounds such as neonicotinoids, being 38 compounds. Gas chromatography was selected to analyze non-thermolabile and volatile compounds - or semi-volatile compounds with a prior derivatization step - being 93 compounds. LC-MS/MS analyses were performed using the MRM mode with 2 mass transitions for each compound, one for quantification and one for qualification. GC-MS/MS analyses were preceded with a thermal desorption step. MRM mode was also used for detection and quantification. For both analytical methods, calibrations were done by spiking within the matrices using deuterated compounds as internal standards. Evaluation of reproducibility, repeatability and extraction yields were also performed with satisfactory results for most compounds. LOD and LOQ were determined as the concentrations giving signal to noise (S/N) ratios of 3 and 10 respectively. In this project, we developed and validated two distinct analytical methods which showed very good analytical characteristics. Those methods were successfully applied to 70 blood samples, 35 plasma samples and 22 feather samples collected in 2021 in the area of La Rochelle, France. The choice of several complementary matrices such as blood, plasma and feathers allowed a good assessment of environmental contaminations for a selected population of breeding birds. This study also showed the importance of biomonitoring combined with such analytical methods for more accurate evaluations of environmental pollution.

3.06.P-Tu148 Volatile Organic Compounds and Their Influence on Tropospheric Ozone Formation in Semi-Urban and Rural Areas

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In order to develop effective pollution control measures, it is essential to know the chemical composition of the atmosphere and the mechanisms of formation of air pollutants. Air pollution is one of the most pressing environmental issues for human health and its occurrence in semi-urban and rural areas cannot be underestimated. While traffic-related compounds are the main pollutants in most urban regions, agricultural phytosanitary compounds and biomass burning products could be the most relevant in semi-urban and rural areas. Besides direct emissions of contaminants to the atmosphere, the impact of air pollution is also related to weather changes. Atmospheric temperature inversion episodes imply stagnant conditions that restrict the dispersion of atmospheric pollutants fostering the transformation of chemical compounds through photooxidation and increasing the concentrations of toxic compounds and human exposure. Unfortunately, the knowledge on the qualitative and quantitative changes in the composition of atmospheric pollutants and their vertical transport under varying weather conditions is very limited and need to be elucidated. The present study is concerned with the vertical distribution of the chemical composition of Volatile Organic Compounds (VOCs) and its influence on O₃ formation within and above the temperature inversion layer in two different areas from Catalonia: Vic (semi-urban) and Mollerussa (rural). The former undergoes frequent temperature inversion conditions and shows one of the highest summer concentrations of ozone of the Iberian Peninsula. The second is a rural area with a high agricultural activity, where the application of phytosanitary products and pesticides can cause the increase of other pollutants like ambient VOCs and O₃. Sampling campaigns were performed in winter and summer to assess the seasonal variations of VOCs on molecular level, including both biogenic (isoprene and monoterpenes) and anthropogenic compounds (alkanes, alkenes, aromatic hydrocarbons and oxygenated compounds). The vertical distribution of VOCs was performed using meteorological balloons fitted with active sampling pumps and different adsorbent cartridges. The chemical analysis included thermal desorption-gas chromatography mass spectrometry (TD-GC-MS) and high-performance liquid chromatography (HPLC) techniques.

3.06.P-Tu149 Industrial Air Emission Impact on Human Exposure and Health - Towards Evidence-Based Decision Making

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Better air quality would significantly improve European population well-being, which is why the European Commission recently launched the Zero Pollution Action Plan “to reduce the health impact of air pollution by more than 55%”. The non-ferrous metal sector (NFM) is committed to continuing lowering its emissions of air pollutants in the most cost-effective and evidence-based approach. This can be achieved by quantifying source/sector specific health impacts and then assessing economic feasibility to reduce emissions from source/sector. Source/sector specific health impact can be quantified by using Burden of Disease (BoD) assessment as recommended by WHO. [1] The aim of the study is to quantify the impact of the NFM industry stack- and fugitive emissions on human exposure and health by quantifying factory emissions attributable fraction to ground level concentration

levels, population attributable exposure fraction and health impact by using the BoD concept. Key questions are: What is the NFM sector emissions impact on population level exposure and health (current situation)? What is the NFM sector emissions relative impact to population exposure and health compared to other sectors (can the NFM sector make a significant change)? What is the NFM sector potential to make air healthier for humans (what can be done)? Answering these questions is used to define what the NFM industry can do to contribute to air quality and identify cost-effective solutions associated with a significant impact on population exposure and health. The approach was tested with a virtual factory placed in a typical industrial site. Health impacts of Cadmium (Cd), Nickel (Ni) and Arsenic (As) metal emissions were evaluated in terms of Disability Adjusted Life Years (DALYs). First results showed that DALYs by Cd, Ni and As factory emissions were low compared to emissions attributable to transportation. The evaluation continues by moving towards the estimation of PM, NO_x and SO₂. The study outcomes will be extrapolated to estimate NFM industry health impact by scaling average emissions with total production volumes and assuming similar average population attributable fraction, and answer the above-mentioned scientific questions. References [1] WHO, 'Health risk assessment of air pollution - general principles.', World Health Organization Regional Office for Europe, Copenhagen, Denmark, 2016.

3.06.P-Tu150 COSMOtherm Estimates of Organophosphate Ester Oxidation Product Physico-Chemical Properties

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Chemicals released into the atmosphere have a finite lifetime, usually ending in transformation into other products by oxidation processes. As a result, it is insufficient to consider the fate of only the initially released chemical, but ultimately necessary to consider the fate and properties of their oxidation products. In this study, we present computational estimates of properties associated with organophosphate ester oxidation reactions, demonstrating the extent of variation in key physico-chemical properties such as partition ratios K_{ow}, K_{aw} and K_{oa}. These results are used to illustrate such variation using chemical space plots of log(K_{aw}) vs log(K_{ow}). In general, oxidation increases the polarity of such chemicals, resulting in increased hydrophilicity and corresponding lowering of K_{aw} and K_{ow}. Thus, oxidation of chemicals in the atmosphere can be seen as a driving force for oxidation products to leave the atmosphere and enter the hydrosphere. As well, propensity for long-range transport and bioaccumulation are lowered.

3.06.P-Tu151 Permeation of Chlorpyrifos in Ex Vivo Pig Skin - Experimental Conditions to Get Closer to Human Exposure

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Exposure to air pollutants is not limited to inhalation risk and dermal contact is gaining increasing interest with the possibility to represent a risky carcinogenic route. Chlorpyrifos is a broad-spectrum organophosphorus pesticide used for aerial and ground-based treatments with different permeation values being reported for human skin. Despite the recommendations of the organisation for economic co-operation and development (OECD) to use 50% of ethanol in the receptor fluid for skin permeation of lipophilic compounds (as chlorpyrifos), this condition was suggested to exaggerate its permeation. Indeed, the definition of the appropriate receptor condition for lipophilic compounds is a common unmet need pointed by different research groups in the area. The aim of this study was to understand the most appropriate receptor fluid composition to be recommended for the permeation of chlorpyrifos in *ex vivo* pig skin that mimics human exposure. For this, several receptor fluid compositions were investigated. Permeation assays were carried with *ex vivo* pig skin mounted on Franz cells and different concentrations of ethanol in the receptor fluid were tested. Permeation assays were carried out at 32 °C and cumulative permeation of chlorpyrifos quantified by HPLC. The results were compared with kinetic parameters reported in the literature for human exposure to chlorpyrifos. Higher quantities of permeated chlorpyrifos are achieved when using the highest concentration of ethanol in the receptor fluid and the values obtained for the permeation kinetic parameters - flux and T_{lag} - are related to the percentage of ethanol in the receptor fluid. Indeed, the permeation flux is directly correlated with the ethanol concentration in the receptor fluid. Finally, when using the lowest concentration of ethanol in the receptor fluid, the permeation flux was 0.63±0.22 µg/cm²/h, which greatly approximates to the value of 0.46 µg/cm²/h reported in human studies. When using *ex vivo* pig skin, the experimental condition that best approximates to kinetic parameters obtained for human skin exposure to chlorpyrifos is the use of lower concentrations of ethanol in the receptor fluid. This work addressed a reported unmet need thereby supporting experimental conditions to be considered in this research field.

3.06.P-Tu152 Reduction of Odor Emitted From Swine Manure Facility by Bio-Foam Cover

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In this pilot-scale study for ammonia (NH₃) reduction from swine facilities, bio-foam was produced by surfactant solution and ammonia-degrading microbes using automatic foam generation unit and sprayed onto the manure surface. We report that the bio-foam spraying significantly suppresses the release of NH₃ from swine manure (located in the four different real swine manure storage facilities in Yongin city, South Korea) irrespective of the seasonal change. The designed biofoam spraying system was as effective as laboratory testing (previous study) for NH₃ masking/degradation and complete coverage of manure was ensured within 40-50 min. The applied biofoam in this study was very dry (Hexagonal, foam quality; 99.0-99.2% and foam density 0.0080 g/mL), medium expansion and stable (half-life; 120 min) with foam bubble density of 25 bubbles/cm² (small size bubbles). Furthermore, this study reduced NH₃ significantly higher (90%±100 %) compared to the control (without treatment; 25%±43 %). Considering this evidence, the bio-foam has two major functions; i.e. biofoam can act as a physical barrier to prevent early release of malodorous compounds into the atmosphere, and it ultimately degrades malodorous (bad odor gases) by the bacteria, after

breaking the foam. Furthermore, surfactant foam can also serve as an effective medium for bacteria to distribute themselves uniformly in the manure pits. (This work was supported by Korea Smart Farm R&D Foundation (Grant 421020-03)).

3.07 Influence of climate change on the fate of persistent organic pollutants and chemicals of emerging concern in the Arctic

3.07.T-01 Assessing Climate Change Influence on Persistent Organic Pollutants (POPs) and Chemicals of Emerging Arctic Concern (CEACs) in the Arctic Physical Environment

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The physical environment in the Arctic has changed significantly as a result of climate change and such changes, e.g. increasing temperatures, sea ice retreat, slumping permafrost, changing sea ice regimes, glacial loss and changes in precipitation patterns, can impact contaminant distribution and subsequently affect the Arctic ecosystems. Evidence of the influence of climate change on contaminant circulation and transport among various Arctic environmental media, including air, ice, snow, permafrost, fresh water and marine environment, are summarized in this presentation. We will broaden the discussion on how climate change may influence contaminant fate in similar cold-climate ecosystems, including Antarctica and the Tibetan Plateau. Indirect effects of climate change on contaminants in the Arctic, including those of extreme weather events, increase in wild fires, and increased human activities leading to new local contaminant sources, have been identified as significant knowledge gaps. Intensified contaminant movements into marine and freshwater ecosystems were observed due to climate change, but better connections are needed with observed effects due to subsequent exposure and accumulation of contaminants in biotic species. Emerging issues, including potential enhanced impacts of higher molecular weight halogenated natural products (hHNPs) and microplastics under a changing climate will be explored and their implications discussed.

3.07.T-02 The Influence of Global Climate Change on Accumulation and Toxicity of Persistent Organic Pollutants and Chemicals of Emerging Arctic Concern in Arctic Food Webs

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As it affects physical, biological, and ecological processes in the environment, global climate change has the potential to influence the uptake and fate of persistent organic pollutants (POPs) and contaminants of emerging Arctic concern (CEACs) in biota and food webs through multiple mechanisms. We summarize the current understanding of how climate change-driven physical and ecological processes influence the levels of persistent organic pollutants (POPs) and contaminants of emerging Arctic concern (CEACs) in biota and food webs within Arctic marine, terrestrial, and freshwater ecosystems. We also address how climate change may interact with other stressors to impact contaminant toxicity and the utility of modelling and newer research tools in closing knowledge gaps on climate change-contaminant interactions. In summary, the study reveals that climate change will impact the long-range transport of pollutants to the Arctic and within the Arctic by altering atmospheric, environmental, and ecological processes, and thus will influence the exposure and accumulation of POPs in Arctic wildlife. In detail; i) physical climate parameters, including climate oscillation indices, precipitation, water salinity, sea ice age, and sea ice quality, show statistical associations with POPs concentrations in multiple Arctic biota. ii) Permafrost thaw is influencing the concentrations of POPs in freshwater ecosystems. iii) Northward range-shifting species can act as biovectors for POPs and CEACs into Arctic marine food webs. iv) Shifts in trophic position can alter POPs concentrations in populations of Arctic species. v) Reductions in body condition are associated with increased levels of POPs in some biota. vi) Models are useful for predicting the net result of various contrasting climate-driven processes on POP and CEAC exposures; however, for some parameters, especially food web changes, insufficient data exists with which to populate such models. In addition to the impact of global regulations on POP levels in Arctic biota, we demonstrate that there are various direct and indirect mechanisms by which climate change can influence contaminant exposure, accumulation, and effects. Therefore, it is important to identify the actual factors that contribute to the variations in POPs to better inform future regulations and policies.

3.07.T-03 Does Climate Change Affect the Long-Term Time Series of Persistent Organic Pollutants (POPs) in Arctic Biota?

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Time series of persistent organic pollutants (POPs) in Arctic wildlife have been established under national monitoring

programmes, generally showing decreasing concentrations of POPs over time, although exceptions exist. These time series are also used to evaluate the effectiveness of international regulations, such as the Stockholm Convention on POPs of the United Nations, regarding decreases in POP concentrations as reflective of reduced primary emissions. However, climate change might affect the fate of POPs in the Arctic, and ultimately the long-term time series, through direct physical and chemical processes or complex ecosystem changes. This study was part of an assessment of the Arctic Monitoring and Assessment Programme (AMAP), analysing links between climate parameters and/or biological variables and the time trends of POPs in Arctic biota, with some additional information from the Antarctic. Several correlative relationships were identified between POP trends in Arctic freshwater and marine biota and climate parameters such as oscillation indices, sea ice extent and its time of formation, temperature and precipitation, but little is known of mechanisms behind these observations. Permafrost thawing seems to lead to increased inputs of POPs into Arctic lake systems. Biological data indicate changes in the diet and trophic level for some species, e.g. thick-billed murres (*Uria lomvia*) and polar bears (*Ursus maritimus*), with consequences for their POP exposure. Studies from the Antarctic suggested associations between iceberg calving and concentrations of POPs in fish, a phenomenon that has not been studied in the Arctic yet. The overall trends of long time series did not change substantially when climate and/or biological parameters were considered, indicating that primary emissions remain the main driver of time trends in Arctic biota. However, rates of annual change could be affected, and local and temporary perturbations occurred in the long-term time series. Effects appeared more pronounced in recent years and often showed a time-lag influence, suggesting that climate-related effects might gain importance in the future. The findings indicate a development towards a more dynamic and complex exposure situation for Arctic biota.

3.07.T-04 Influence of Climate Change on Persistent Organic Pollutants and Chemicals of Emerging Arctic Concern: Overview of the State of Knowledge

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A recent Arctic Monitoring and Assessment Programme (AMAP) report addresses the influence of climate change on persistent organic pollutants (POPs) and chemicals of emerging Arctic concern (CEACs) based on empirical data available in the past 10 years. Higher temperatures are leading to release of stored POPs from melting ice, glaciers and thawing permafrost into aquatic systems, and are also affecting the uptake and elimination of POPs in cold-blooded organisms. Reductions in the amount and extent of sea ice are influencing air-water exchange of contaminants and prey accessibility. Northward movement of subarctic species from the Atlantic and Pacific leads to increased prey-switching, affecting POP exposure and tissue levels. Increasing temperatures and cryospheric changes have increased the mobility and transfer of POPs between physical environmental compartments of the Arctic through various mechanisms. Climate change and a diminishing cryosphere will likely increase human activity in the Arctic, including shipping, tourism, and industrial operations, as well as promote the expansion of urban areas, contributing to local releases of POPs and CEACs. Temporal trends of POPs in the Arctic are used in effectiveness evaluations of the Stockholm Convention, which regulates primary emissions of POPs. POP concentrations have generally decreased in the Arctic environment and biota, but some POP time series have shown recent perturbations in these decreasing trends associated with climate-related changes in physical processes or ecosystems. Variations in climate oscillation indices, which reflect changes in air mass movements, ocean currents, and thus contaminant transport, are correlated with changes in POP accumulation in some Arctic biota. Temporal associations between POP concentrations in air and biota and climate parameters (e.g. climate oscillation indices, sea ice extent, precipitation) are also seen. The reduction of primary emissions is still considered to be the main driver of POP time trends, however, for some compounds, the rate of concentration decline has been correlated (negatively or positively) with climate-related parameters. Although the assessment shows climate-change related influences on POPs and CEACs, considerable knowledge gaps exist and further monitoring and research are needed to fill these. The coordinated research and monitoring approach under AMAP will help provide new data to evaluate these emerging climate-contaminant issues.

3.07 Influence of climate change on the fate of persistent organic pollutants and chemicals of emerging concern in the Arctic (Virtual Only)

3.07.V-01 "Nothin' Like a DAME": Biogeochemistry of Drosophilin a Methyl Ether and Other Halomethoxybenzenes in the Nordic Region

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Drosophilin A (DA: 2,3,5,6-tetrachloro-4-methoxyphenol), drosophilin A methyl ether (DAME: 1,2,4,5-tetrachloro-3,6-dimethoxybenzene), and chlorinated anisoles are halogenated natural products (HNPs) of terrestrial fungi, particularly basidiomycetes. Tetrachloroveratrole (TeCV: 1,2,3,4-tetrachloro-5,6-dimethoxybenzene), along with DiCV and TriCV, are metabolites of chloroguaicols in bleached kraft mill effluent. We searched the Nordic ecosystem for these chloromethoxybenzenes, as well as for bromoanisoles (BAs) of marine origin. The sample set comprised air and atmospheric deposition from southern Sweden, air from Arctic Finland, rivers flowing into the northern Baltic, small Baltic estuaries, terrestrial fungi and forest litter. DAME and BAs were prominent in air, deposition, rivers and estuaries, while TeCV and pentachloroanisole (PeCA) was less abundant or absent. DAME in terrestrial fungi were 400 and 900 ng/g fresh weight in two species, 2-7 ng/g in eight species, and < 1 ng/g in sixteen species. Four samples of forest litter contained 2, 57, 68 and 900 ng/g of DAME. In estuaries, DAME was negatively correlated with salinity and BAs, and positively correlated with dissolved organic carbon, which is high in northern Baltic streams. These observations suggest that DAME is not produced within the estuaries, but

is of terrestrial origin. Fungi and forest litter containing fungal mycelia are suggested as sources to the atmosphere by volatilization, and to estuaries by precipitation, streams, surface runoff and possibly submarine groundwater discharge. Production and biogeochemical cycles of marine brominated HNP are influenced by climate change, and we suggest similar influence for DAME and other terrestrial HNPs.

3.07.V-02 Climate and Weather Factors of Influence on the Temporal Trends of Concentrations of Highly Bioaccumulative POPs in Seabird Eggs and Polar Bears From the Hudson Bay, Canada

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Persistent organic pollutants (POPs) are found throughout the Arctic environment including in wildlife and in Northern people, and are monitored extensively in Northern Canada in order to assess temporal changes in concentrations. Incorporating climate or weather variables into temporal models of POP levels can identify consistent variables of influence and can also strengthen temporal components of the models. The present study investigated the comparative time series of concentrations of five bioaccumulative POPs in polar bear (*Ursus maritimus*) tissues (fat, liver) and thick-billed murre (*Uria lomvia*) eggs from Hudson Bay populations. Temporal trends were analyzed using log-linear regressions and multivariate general linear models with year and weather/climate variables. Log-linear regressions showed that *p,p'*-dichlorodiphenyltrichloroethane (DDT) and 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) decreased significantly in murre eggs (1993–2015), though 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) increased slowly (1.6 % per year). γ -hexachlorocyclohexane (γ -HCH) and *p,p'*- DDT also decreased in polar bear fat from the Western Hudson Bay (WHB; 1991–2015), though BDE-47 increased relatively rapidly (9.6 % per year). None of the POPs showed significant trends in Southern Hudson Bay (SHB) polar bears (2007–2016), and perfluorooctane sulfonate (PFOS) did not change significantly in liver of WHB or SHB bears. Time-lagged Arctic and North Atlantic Oscillation indices (AO and NAO) were consistently related to POP levels in both species; sea ice freeze up dates, length of the ice free periods, air temperatures and wind speeds also produced consistently significant models. Relationship directions varied by season and time-lag length, sometimes within the same year, consistent with previous studies. Adding weather or climate variables improved the relationships with “year” in several multivariate models, producing interpretable temporal relationships from previously insignificant ones. E.g., In a multivariate model with 1-year time-lagged June AO, γ -HCH decreased significantly in murre eggs while γ -HCH and CB-153 also decreased significantly in SHB bear multivariate models. Some consistent climate and weather factors of influence have now been identified through this and similar studies, and these can improve temporal analyses of POPs levels. However, continued re-analysis as these time series is needed to deduce consistent directional change relationships.

3.07.V-03 Long-Range Transport and Temporal Trends of Emerging Organic Contaminants in the Arctic - Impacted by Human Activities and Climate Change

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Emerging organic contaminants (EOCs) may reach ecologically sensitive Arctic environment via atmospheric and/or oceanic long range transport. They are subject to a variety of processes in the Arctic environment such as degradation, bioaccumulation and interaction between the atmosphere, snow, water and soil. Additionally, climate change may significantly influence the transport and environment fate of EOCs in the Arctic. As a part of collaborative German-French program at joint French-German Arctic Research Base (AWIPEV) in Ny-Alesund, Svalbard, this project is proposed to investigate the occurrence and long term trends of EOCs in Arctic air, water and snow. Integrated high-volume air samples were taken on the platform of German Atmospheric Observatory using a high-volume pump operated for 7 days to obtain a volume of ~2500 m³. A glass fiber filter is used to trap the airborne particles and the gaseous contaminants are collected with a PUF/XAD-2 resin column. Surface snow samples were collected on the glaciers around Ny-Alesund and seawater samples were obtained in Kongs Fjord from 2011 to 2018. EOCs including poly- and perfluoro alkyl substances (PFASs), brominated flame retardants (BFRs) and organophosphate esters (OPEs) have been determined in all air, seawater and snow samples. Data achieved from this study may improve models to predict the environmental progression and assess the effect of human activities and climate change on remobilization and phase exchange for EOCs in the Arctic ecosystem.

3.07.V-04 Temporal Trends of Persistent Organic Pollutants in a High Arctic Seabird Over 15 Years: Influenced by Climate Variability and Dietary Plasticity?

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Global change is currently inducing drastic changes in the Arctic ecosystem, which are highly suspected to ultimately impact contaminant exposure and health risks in Arctic wildlife. The dynamic of contaminant exposure over time can be monitored through the bioaccumulation in top predators being considered as relevant sentinels of environmental pollution. Long-lived apex species, such as the black-legged kittiwake (*Rissa tridactyla*) from the Norwegian Arctic, is chronically exposed to a complex cocktail of harmful contaminants. In this study, we describe and assess long-term trends of a wide range of legacy POPs in

Svalbard kittiwakes over 15 years (2007-2021), based on a unique collection of blood samples collected during the chick-rearing period in males and females (n=500). We also identify how these time trends may be impacted by biological factors (i.e., morphometry, body condition and sex), dietary ecology (inferred from stable isotope and stomach content analysis), and climate (climate teleconnections and local conditions), and oceanographic conditions in shaping the magnitude and profile of POP exposure in Svalbard kittiwakes. We were able to depict temporal trends of a wide range of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). Preliminary results indicate overall stable or decreasing trends for most of the POPs, likely in response to reduced primary emissions. PCBs and OCPs have been regulated during the 1970s by national bans and were followed by international regulations under the Stockholm Convention of POPs, which entered into force in 2004. Interestingly, a few notable exceptions were pointed out, such as for p,p'-DDE and oxychlorodane, which appear to increase in blood of kittiwakes over the studied period. In recent years, Kongsfjorden has been strongly influenced by the West Spitsbergen Current with increasing temperature, declining sea ice cover and prey community shifting with periodic arrivals of Atlantic species. There is also a considerable annual variation in the dietary composition and niche of the kittiwakes, both evidenced by spontaneous regurgitates as well as by stable carbon and nitrogen isotopes. Consequently, both climate variability and dietary plasticity could impact temporal trends of POP exposure in kittiwakes. Specifically, a shift in prey community associated to secondary emissions in response to melting sea ice due to climate warming may lead to increasing bioaccumulation of some POPs in Arctic kittiwakes.

3.07.V-05 Understanding the Fate of Microplastics in Sea Ice

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In the Arctic, microplastic pollution has the potential to be a major threat. Although very few studies have been carried out thus far, their presence has already been discovered in sea ice, snow, surface and sub-surface waters and deep-sea sediment, as well as Arctic biota. Arctic sea ice has been recognised as a potential sink and mode of transport for microplastic particles. The purpose of this experiment was to provide a mechanistic understanding of plastic particle uptake and behaviour in forming sea ice using the Roland von Glasow Air-Sea-Ice Chamber (RvG-ASIC) at the University of East Anglia, UK. Applying polyethylene spheres (ranging ~50 – 300mm dia) to the chamber sea water, preliminary results show sea ice formation results in the entrainment of these microplastics into the ice. The highest particle concentrations were observed in the uppermost layer of sea ice (ice – atmosphere interface), with lower particle concentrations in the base of the ice and the surrounding seawater (surface and sub-surface). Microplastic release during simulated sea ice thaw was also tracked, with evidence of particle accumulation in a buoyant, lower-saline meltwater layer beneath the ice. The findings from this study provide insight into the accumulation and fate of relatively buoyant microplastic particles in sea ice. In turn, these will serve to improve oceanic modelling approaches to predict microplastic fate in ice-covered regions.

3.07 Influence of climate change on the fate of persistent organic pollutants and chemicals of emerging concern in the Arctic (Poster)

3.07.P-Mo139 Poly- and Perfluoroalkyl Substances (PFAS) in a Firn Core From Austfonna, Svalbard

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In spring 2015 we drilled a 13.2 m deep firn core, representing years mid-2001 to early-2015, from Austfonna, the largest ice cap on Svalbard. The core was shipped frozen to the Norwegian Polar Institute in Tromsø where we separated it based on density into 18 samples of sufficient volume for analysis of 40 PFAS compounds in 9 groups, including perfluoroalkyl carboxylic acids, perfluoroalkyl sulfonic acids, fluorotelomer sulfonic acids, perfluorooctane sulfonamides, perfluorooctane sulfonamide ethanols, and perfluorooctane sulfonamidoacetic acids, per and polyfluoroether carboxylic acids, ether sulfonic acids, and fluorotelomer carboxylic acids, using USEPA Method 1633 (draft). The results show that 5 of the 40 PFAS compounds appear in at least one sample, mostly close to the detection limit. The most often observed was PFOA in 11 samples, 8:2 FTS in 5 samples, PFNA in 4 samples, and one observation each of PFOS and PFHpA. The most concentrated of any PFAS was consistently 8:2 FTS, with range of 1.48 – 2.77 ng L⁻¹, the latter in a sample from mid-2001 to early-2002. The appearance of 8:2 FTS is unusual because it is considered to be a biological decomposition intermediate that can further decompose to PFOA. Its origin at Austfonna is not clear. PFOA appeared the 6 surface samples from early-2011 to early-2015, and 5 deeper samples as early as mid-2001. PFOA concentrations range nearly a factor of 2 from 0.386 ng L⁻¹ to 0.744 ng L⁻¹, but there is no trend in the results: the deepest sample from mid-2001 was 0.592 ng L⁻¹ and the sample, from 13 years later was 0.553 ng L⁻¹. We assume that the source of PFOA and PFNA is oxidation of 8:2 FTOH, a volatile PFAS found in comparatively high concentrations in Ny-Ålesund air in 2013, with an 80-day atmospheric residence time. PFNA, had concentration ranging from 0.381 ng L⁻¹ from late-2013 to late-2014 up to 0.572 ng L⁻¹ from late-2003 to late-2004. The PFNA amounts, when found with PFOA, are similar, suggesting that both are oxidation products of 8:2 FTOH. However, atmospheric oxidation on Svalbard is limited to periods of UV-B input from late February to late October. PFOS was found once in the sample from late-2003 to late-2004, suggesting that it is not abundant in the Svalbard atmosphere. PFHpA, was found once from mid-2001 to early-2002. The results suggest that atmospheric deposition of PFAS at Austfonna is limited, largely to oxidation products, and to 8:2 FTS.

3.07.P-Mo140 POPs and Climate Change in Antarctic Ecosystems From a Bi-Polar Perspective

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The climate crisis is affecting Antarctica and the Southern Ocean and effects have been already reported for the abiotic compartments of the ecosystems, e.g. ice loss and iceberg calving. The global warming can alter also the distribution of persistent organic pollutant (POPs) both at a global scale and in the Antarctic Region, due to their physical-chemical characteristics. Effects of climate changes have been already reported on feeding behavior and reproductive process of organisms. Another consequence for organisms includes the POP bioaccumulation. Here we review the literature reporting the linkage between recorded effects of climate changes and POP bioaccumulation in resident marine Antarctic species (fish and penguins). We focused our data revision on PCBs, *p,p'*-DDT, *p,p'*-DDE, DDTs, and HCB since other chemicals, including emergent contaminants, were reported only in very few articles not allowing any comparison. Notwithstanding Antarctica is a final sink for persistent contaminants due to the extreme cold climate, a general decreasing POP trend has been observed for some POPs, although they seem weak and inconstant. Their concentrations in marine biota are reported to be linked to ice melting and large iceberg calving; these effects have not been studied in the Arctic to any significant degree, despite they are likely to occur given the similarities of polar environments. The comparison with the Arctic biota is not always possible due to the lack of data in the Antarctic Region, both for terrestrial and marine ecosystems. The climate-driven changes may affect the marine Antarctic ecosystems, where the peculiar pelagic-benthic coupling may also contribute to alterations in the bioaccumulation processes. The peculiar ecosystems and trophic web structure and functioning make Antarctic biota vulnerable to effects of persistent contaminants; the impact of climate-driven environmental changes on POP bioaccumulation processes in marine organisms, not only top predators, is still to be understood. It remains an open question if the POP amount accumulated in the Antarctic ecosystems is decreasing or not depending on the variation of climate parameters.

3.07.P-Mo141 Influence of Climate on Long Term Trends of Brominated Flame Retardants and Perfluorinated Alkyl Substances in Landlocked Arctic Char in the Canadian Arctic

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Landlocked Arctic char are the only top predators in most high Arctic lakes and they can serve as a sentinel species for changes in atmospheric inputs of persistent organic pollutants (POPs) to remote freshwater environments. Previous work has shown that PCBs and other legacy POPs have been declining in Arctic air and in landlocked char from isolated lakes in the Canadian Arctic over the past 20 years. However, atmospheric measurements and studies of ice cap snow/firn/ice cores published over the past 10 years have shown continuing and, in some cases, increasing, inputs of polybromodiphenyl ethers (PBDEs) and poly/perfluoroalkyl substances (PFAS) to the high Arctic. Therefore, we were interested to see if these trends were reflected in the landlocked char as well. PBDEs (13 Br3-Br7 congeners), C6-C14-perfluoro carboxylates (?PFCAs) and perfluorooctane sulfonate (PFOS) were determined in muscle of Arctic char and compared results to published global emission estimates and to climate related parameters such as glacier runoff, air temperatures, summer ice free area of the lakes. Data was available for char from 4 lakes (Resolute, Char, Amituk and Hazen), spanning 12 to 19 sampling years from the early-1990s to 2019, with sample sizes ranging from 3 to 10 fish per year. ¹³PBDEs significantly increased in Char, Resolute and Hazen from the 1990s to 2017 (6.6 to 9.5%/y). The increase of PBDEs contrasts with phase out of PBDEs in the mid-2000s and modelled predictions of declining global emissions. ⁷C₇-C₁₄-PFCAs generally declined from 2007/08 to 2015 in all lakes and then showed increasing concentrations in the two most remote lakes in Hazen and Amituk (~ 40% /y) over the period 2015-2019. The increasing trends of ?PFCAs and PFOS in char were surprising given the phase-out of perfluorooctanoic acid and related PFAS by 2014 as well as PFOS in the early 2000s. Nevertheless, the general trends of ?PFCAs and PFOS in char are in agreement with published snow/ice and air measurements from the mid-2000s to 2015. Climate related factors could be important. PFOS in char was positively correlated with increasing glacial runoff in Lake Hazen while ¹³PBDEs were not. Annual summer ice free area or ice out date was not correlated with year to year variation of the PBDEs or PFAS. Further assessment of the influence of climate warming would be aided by having a more sampling years in order to increase statistical power as well as local rather than regional climate data.

3.07.P-Mo142 Influence of Climate Related Factors on the Temporal Trends of PCBs and Perfluoroalkyl Substances in Landlocked Char in Two High Arctic Lakes

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Temporal trends and climate related parameters affecting the fate of polychlorinated biphenyls (PCBs) and perfluoroalkyl substances (PFASs) were examined in landlocked Arctic char (*Salvelinus alpinus*) in two paired lakes, West and East lakes, on Melville Island in the Canadian High Arctic. Research over the past 15 years in the study area has revealed ongoing permafrost disturbances in the West Lake watershed and sub-aqueous sediment slumps in the lake, which have impacted lake turbidity. Adult char were collected in late July from 2008 to 2019, by gill netting. In total, more than 140 samples of char were collected and muscle analyzed for PCBs and PFASs. The study goals were to i) examine temporal trends of two classes of POPs differing in physical-chemical properties, ii) study which parameters are affecting the occurrence of these POPs in Arctic char, and iii) investigate the influence of climatic disturbances affecting the lake catchments, water chemistry, and fish growth rates, on the temporal trends of hydrophobic PCBs and hydrophilic PFASs. ⁷¹PCB (lipid adjusted) showed a declining trend in Arctic char from East Lake (-7.4 % /yr; P=0.058, 2008-19) as expected due to the past national and regional bans/ restrictions on use and emissions of these chemicals. However, PCBs were higher in char from West Lake and increased significantly from 2008 to 2015 (+10 % /yr; P=0.02, 2008-15). PCB concentrations declined in from their peak in 2015 but remained higher than in East Lake char in 2017. The increases of PCBs in char from West Lake in comparison to East Lake coincided with a large increase in lake

turbidity. The lake is undergoing greater inputs of PCBs bound to terrestrial carbon, due to permafrost disturbances, as well as to mobilization of PCBs from sediments into the water column during subaqueous slumps. Higher growth rates of East Lake char may also have contributed to the observed decline. On the other hand, total (C4-C14) perfluorocarboxylates (PFCAs) concentrations in char showed a decreasing (but non-statistically significant) trend of 8%/y in West Lake and 3%/y in East Lake over the period 2008 to 2019. Concentrations of PFASs in West and East lakes were similar in char muscle, despite major differences in water chemistry including turbidity. These results suggest that climate disturbances due to warming may affect, in different magnitudes, the exposure of Arctic freshwater biota to hydrophobic and hydrophilic POPs.

3.07.P-Mo143 Inputs of Organochlorine Pesticides, Organohalogen Industrial Compounds and Brominated Flame Retardants to Annual Surface Snow on the Juneau Icefield

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In June 2006 we collected bulk annual surface snow from two sites on the Juneau Icefield (JIF), which straddles the Alaska-British Columbia border and is experiencing warming air temperatures and glacial loss. Samples were collected about 3 km apart and 366 m different elevation, at Summit (1646 m a.s.l., Lat 55.560666N, Long 134.408344W) and at North Branch (1280 m a.s.l., Lat 58.54577778N, Long 134.456139W). We analyzed the samples for 23 organochlorine pesticides (OCPs) and 16 organohalogen industrial compounds (OHICs, mostly chloro- and bromo-benzenes) and 19 brominated flame retardants (BFRs). Total OCP burden at Summit is 377 pg L⁻¹ while at North Branch it is 225 pg L⁻¹. Most of the difference is accounted for by a much higher amount of β -endosulfan at Summit. OCP maximum amount was 50.5 pg L⁻¹ for endrin at Summit. Total OHIC burden at Summit is 32940 pg L⁻¹, while at North Branch it is slightly higher at 34160 pg L⁻¹. These high values result from the high amounts of three dichlorobenzenes (diCBs) which peaked at 26600 pg L⁻¹ for 1,4-diCB at Summit. The 3 diCBs, 1,2-diCB, 1,3-diCB and 1,4-diCB comprise 99% of all OHIC at both Summit and North Branch. Without the diCBs, the OHIC burden at Summit is 303 pg L⁻¹ and at North Branch it is 397 pg L⁻¹, values more comparable to those of OCPs. The higher value at North Branch is again due to several of the non diCB OHICs having higher concentrations there. The comparatively high concentrations of the diCBs is typical in snow and ice in our earlier work on the Antarctic Plateau (2001) and at the Holtedahlfonna glacier (2005) on Svalbard. The OHIC results from JIF are consistent with earlier research, suggesting that these comparatively volatile contaminants are found throughout the global atmosphere at concentrations higher than most other chlorinated or brominated contaminants. They are known to be high production chemicals in the United States since the early 1920s. The total BFR burden at Summit is 223 pg L⁻¹, while at North Branch it is 617 pg L⁻¹ and is a result of most BFRs at higher concentrations at North Branch except polybrominated diphenyl ether (PBDE)-17, PBDE-28, 1,2-Bis(2,4,6-tribromophenoxy) ethane and pentabromo ethylbenzene. The maximum amount for an individual BFR was 312 pg L⁻¹ for PBDE-99 at North Branch. Clearly there is a different source at North Branch. The results for OCPs and BFRs suggest the influence of different sources at these sites.

3.07.P-Mo144 Chlorinated Paraffins in Polar Cod and Capelin From the Barents Sea During the Arctic Spring Bloom

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Chlorinated paraffins (CPs) are currently under intense regulatory scrutiny due to their widespread distribution in the environment and are listed as contaminants of emerging Arctic concern. As of 2017, short-chain chlorinated paraffins (SCCPs) have been added under the Stockholm Convention to restrict their production and use due to their long-range transport (LRT) potential, bio-accumulative and toxic properties. This highlights the need for additional data from the Arctic regions, which is understudied due to their challenges in trace level analysis of these compounds. Many Arctic animals have utilized their main energy reserves by March, before the onset of the spring bloom and upon return of the sun. By May, the spring bloom reaches its peak, and energy generated therein propagates through the entire ecosystem. With ongoing climate change, food web dynamics might change, and with that CP dynamics. However, little is known about seasonal partitioning and transfer of these emerging contaminants, making it challenging to understand and predict climatic change impacts. Two key species in the Arctic marine food web are Polar cod and capelin. They channel energy generated by lower trophic levels like phytoplankton and zooplankton to higher trophic levels like seals and sea birds. Here, we analyzed short-chain and medium chain chlorinated paraffins (SCCPs and MCCPs, respectively) in two key Barents Sea fish species, Polar cod, and capelin in March and May 2021. Preliminary results revealed similar CP levels between species and seasons. We observed dominant SCCP concentrations in Polar cod, whereas MCCPs dominate in capelin, suggesting that capelin acts as biovector for contaminants like CPs to Arctic waters.

3.08 Measuring, monitoring and modelling of pesticide fate and mitigation in a regulatory context

3.08.T-01 How Transparency of Pesticide Use Helps Farmers Meet Compliance Expectations and Introduces Realism in Risk Assessment: The CropLife Europe Digital Label Compliance Project

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The use of Plant Protection Products (pesticides) in agriculture is regulated through EC Regulation No. 1107/2009 that imposes preliminary risk assessments and the precise setting of directions of use and risk mitigation measures for the active substance at

EU level followed by the Plant Protection Product approval at EU member state level before placing them on the market (EC, 2009). The risk assessments rely on hundreds of guideline compliant studies and are performed to reflect worst-case use and exposure scenarios (EC, 2013a and 2013b). Directions for use and all precautions to be taken at all stages must be indicated on the label, thus making labels increasingly more complex to read. Label instructions are established based on the outcome of these worst-case scenarios considered for the risk assessments and thus rarely reflect the conditions under which products are actually used in the field. These reflect the diversity of application machinery, agro-environmental conditions and pest pressures faced by European farmers. Furthermore, modern technologies and stewardship measures are a central piece of a developing a more sustainable food production model as outlined by the EC's Communication on the EU Green Deal and specifically in the F2F and the Biodiversity strategies, reflecting consumers', policymakers' and society's expectations. This presentation will introduce the Digital Label Compliance (DLC) concept developed by CropLife Europe. The DLC aims to de-risk growers and reduce complexity in dealing with the handling and application of Plant Protection Products by increasing label comprehension via digital labels. The automated application of Plant Protection Products according to the digital label considering the geolocation and conditions of a growers field and the subsequent recording of spray operations is maintaining a high-level of protection for human health and the environment whilst de-risking growers.

3.08.T-02 Using GIS Overlay Methods to Determine Vulnerable Agricultural Areas in Europe

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GIS enables the use of meta-models, weighted-overlay, and index methods are frequently used to assess the relative vulnerability of groundwater or surface water to contaminants. The application of these methods is driven in large part by data availability, and assessor expertise and familiarity. This may result in a bias in the models as certain criteria are over- or underemphasized; for example, pesticide behavior is often ignored in commonly used index methods, where environmental factors such as pH and organic carbon have been shown to affect the local vulnerability of groundwater and surface water. Furthermore, vulnerability index methods may feature subjective weights and rankings, which increases the likelihood of bias. Two overlay index methods were developed, applied, and tested to determine groundwater and surface water vulnerability for maize production areas in the Ukraine. Both methods follow a standardized approach in which the applicable data within the areas of interest are ranked and grouped using a cumulative distribution function. A weighting schema was developed by grouping the data in six classes with each class has fixed probability range. The classes were assigned weights, ranging from 1 to 6, with 1 being the lowest weight and 6 being the highest weight. Using this approach, the maximum vulnerability score is 36 and the results show the relative vulnerability for both groundwater and surface water. This approach has the advantage that the distribution of variables is accounted for and are unbiased. For groundwater, the following parameters were included: average annual rainfall, topsoil sand content, topsoil organic carbon content, topsoil pH, drainage class and depth to groundwater. For surface water, key variables included slope, days with more than 25.4 mm of rainfall, topsoil available water capacity, topsoil organic carbon content, topsoil pH and drainage class. The maize production areas falling in the most vulnerable category, aka having the highest score, represent 12% to 13% of the areas of interest. The maximum attainable vulnerable score of 36 was not achieved.

3.08.T-03 Degradation Kinetics on the Next Level

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The current procedure for deriving endpoints from chemical degradation data is based on separate evaluation of the available datasets and subsequent averaging of the parameters obtained. For each individual dataset, the most suitable degradation model is then selected based on statistical criteria and visual evaluation. In many cases the decision requires expert judgement, which frequently leads to discussions with an unpredictable outcome. In this contribution, we show simultaneous evaluations of all available datasets based on nonlinear mixed-effects models and highlight some of the advantages of this method with respect to regulatory degradation kinetics, namely a) the shift of the model selection step to the level of the complete available degradation data, and b) the improved handling of datasets in which certain kinetic parameters, such as the degradation rate constant of a certain metabolite, are ill-defined and where current guidance recommends to use a surrogate value such as a half-life of 1000 days. For illustrating the application of nonlinear mixed-effects models to chemical degradation data, synthetically generated data as well as experimental data were used. For each variant of synthetic datasets, degradation parameters were drawn from a specified distribution of degradation model parameters. In addition, a random noise component was added to the simulated data, with properties similar to the two-component random noise often observed in real datasets. Experimental datasets were obtained from regulatory dossiers published by the European Food Safety Authority (EFSA) on their website. The results presented in this contribution demonstrate that the application of nonlinear mixed-effects models to chemical degradation data is technically feasible. Results obtained for synthetically generated data suggest that this method provides more reliable and accurate representations of the information in the available data. The application of nonlinear mixed-effects models to experimental data shows that the use of surrogate values for previously ill-defined fits can be circumvented. [1] Ranke J, Wöltjen J, Schmidt J, Comet, E. 2021. Taking Kinetic Evaluations of Degradation Data to the Next Level with Nonlinear Mixed-Effects Models. *Environments* 8(8):71.

3.08.T-04 Size Matters: The Effect of Scale in Chemical Biodegradation Studies

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Environmental persistence of synthetic chemicals is a significant ecological challenge for the industry and the public. However, current regulatory tests lack environmental realism, particularly when biodegradation of chemicals is assessed in a very small-scale laboratory systems and the results are then extrapolated to the real world. In this study, we hypothesised that the factor of scale plays a major role in the inconsistency of the rate of degradation between lab-based regulatory testing and field trials. For this study “OECD 308 Aerobic and Anaerobic Transformation in Aquatic Sediment” test guidelines were used to represent regulatory study microcosms ($v = 0.25$ l) and set a comparison with much larger flumes ($v = 25$ l). Five pesticides were used with different chemical properties, including a carboxamide, azole, benzoylurea and two triazine based compounds with a range of Log K_{ow} between 0.069 and 5.12, molecular masses between 166.18 and 511.1 and dissimilar degradation kinetics. We also studied how system scale affected the broader physico-chemical environment including rates of nitrogen and phosphorus biogeochemical cycling processes. The chemical degradation rates were found to be faster in larger test vessels compared to small, with decay rates significantly different with 4 out of 5 chemicals. A Single First-Order kinetics model was used to estimate half-life (DT_{50}) of chemicals in both systems and the DT_{50} of each chemical was found to be significantly shorter in flumes than in microcosms. Additionally, it was found that the biogeochemistry of larger systems was significantly more stable and consistent than smaller microcosms. Not only was the extent of variation over time much higher in smaller vessels, but the variability between replicates was also always higher than in flumes. Microcosms also showed significantly higher bacterial cell count, lower redox potential and oxygen levels and higher ammonium concentration than flumes. This study showed that the size of test system affects biogeochemical cycling in the incubation vessels, underlying microbial community and the rate of degradation of chemicals. The effect of scale also has an impact on test system stability and variation. To understand the effect of scale on microbial ecology, metagenetic analysis for ecological drift, diversity and relative abundance is necessary.

3.08 Measuring, monitoring and modelling of pesticide fate and mitigation in a regulatory context (Virtual Only)

3.08.V-01 Opportunities for Regulatory Decision Making Through Digital Agriculture: A Mapping Exercise

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Crop Protection products are highly regulated in the EU. They are subject to thorough risk assessments following guidance developed by Member States and EFSA. These risk assessments rely on exposure estimates which reflect so called realistic worst case situations as well as increasingly conservative toxicity endpoints. The reality of environmental conditions, agronomic situations and hence the realities under which crop protection products are applied are much more diverse. The rapid developments of digital tools in agriculture now enable us to overlay existing data with in-situ measured data and model predictions. At the same time we observe an increasing pace in the development of hardware, i.e. precision application equipment for crop protection products. Both developments combined allow much more targeted applications of crop protection products, i.e. to apply crop protection products at the most appropriate application rate only where needed, exactly when needed. Machine readable digital labels will enable the computer aided planning, execution and documentation of applications, overlaying the spatial and agronomic information with the approved application conditions of the specific product as described on the product label (e.g. risk mitigation measures). With all this information, assessments of the risk to consumers, the environment or operators, workers and bystanders specific to location, time and available application equipment will be possible. EU regulators and industry have started considerations how to include these new opportunities the digitization of agriculture offers into regulatory decision making. Crop Life Europe has conducted a mapping of these activities across EU institutions, Member States and industry associations. An analysis of analogies and differences is presented as well as an identification of gaps where bespoke risk assessments should be developed.

3.08.V-02 The Casanova Drift Model (CDM): An Arable Crop Spray Drift Deposition Model

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A mechanistic arable crop spray drift model is presented. The model, written in C++ with a Shiny R interface, is a mixture of ballistic tracking of droplets (Lagrangian transport of water droplets through air in time) combined with equations dealing with droplet evaporation, a representation of the horizontal wind profile (from two heights and variation from perpendicular), an approximation of canopy effects, a droplet size distribution (temporal measurement and using a minimum drop size of 18 μ m), and a simplified description of spray sheet breakup. The model has been calibrated and validated against Bayer (internal) USA field data but also European field data from Belgium, France, and Poland. Relative performance (i.e., relative changes in drift due to operational variables such as wind speed) and absolute performance (i.e., modelled drift curves that match results on the ground) have been assessed and will be presented. The model includes modules that may help address issues raised during the SETAC Drift Risk Assessment Workshops (e.g., sampler capture efficiency; locale). The model will be released after the 2022 meeting as publicly available code and interface, along with a nozzle droplet spectra library for standard flat fans commonly used in drift trials across the EU.

3.08 Measuring, monitoring and modelling of pesticide fate and mitigation in a regulatory context (Poster)

3.08.P-Th052 Amended Soil With Different Application Rates of Sugarcane Straw Biochar Interfere Sorption and Desorption of Metribuzin

Kassio Mendes, Kamila Mielke, Maura Gabriela Brochado, Alessandro Lima, Bruna Medeiros and Vanessa Freitas, Federal University of Viçosa, Brazil

Biochar is a carbon-rich material that comes from the partial carbonization of different feedstock under controlled conditions. The use of biochar has been evaluated as an alternative to improve the chemical, physical and biological quality of the soil. However, the application of this material to the soil has shown to directly influence the sorption, desorption, leaching and degradation of pre-emergence herbicides. The effects of biochar on herbicide behavior in soil depend directly on the type of feedstock, pyrolysis temperature and rate of application in the field. Furthermore, herbicides can behave differently depending on the physicochemical characteristics of the molecule. Therefore, the objective of the study was to investigate the sorption and desorption of metribuzin in an amended soil with different application rates of sugarcane straw biochar. The experimental design was entirely randomized with seven application rates of biochar (0, 0.1, 0.5, 1, 1.5, 5, and 10% w w⁻¹) produced in a pyrolysis temperature of 750°C. The sorption and desorption study were carried out in an equilibrium batch method with five concentrations of metribuzin (0.5, 1, 2, 4, and 8mg L⁻¹) where the average concentration corresponds to the highest recommended dose of the herbicide (1920g a.i. ha⁻¹) for sugarcane cultivation. The analysis of the herbicide was performed in High Performance Liquid Chromatography (HPLC). The sorption and desorption coefficient data were fitted to the Freundlich isotherm model (K_f). The K_f values of sorption (1.42mg^(1/21/n) L^{1/n} Kg⁻¹) and desorption (0.78mg^(1/21/n) L^{1/n} Kg⁻¹) were low in unamended soil compared to all amended soils. Application rates of 5 and 10% of biochar increased 6- and 10-fold the K_f values of sorption and decreased desorption between 15- and 45-fold relative to unamended soil. Application rates < 1.5% had no impact on sorption (K_f = 1.84mg^(1/21/n) L^{1/n} Kg⁻¹) and low interference with desorption (2.84mg^(1/21/n) L^{1/n} Kg⁻¹) of metribuzin. The biochar applied at 5 and 10% sorbed 77.8 and 89.4% and desorbed 14.2 and 3.7%, respectively, relative to the initially applied herbicide. The degree of linearity of sorption and desorption (1/n) of the unamended soil averaged 0.59 and varied from 1.0 to 0.4 with increasing biochar application rate. The application of rates >1.5% of sugarcane straw biochar strongly increased sorption and decreased desorption of metribuzin in the soil and can influence the residual effect and weed control.

3.08.P-Th053 Collation and Analysis of Public Environmental Monitoring Datasets for Regulatory Submissions

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For the authorization of plant protection products (PPP) under regulation 1107/2009/EC an overview and assessment of environmental monitoring data must be provided by the applicant. However, there are currently no guidelines for what data should be supplied or how the data should be processed, analysed, presented and interpreted. Data collection and analysis to meet this regulatory requirement draws on existing guidelines, guidance and directives e.g., FOCUSgw (EC, 2014), groundwater monitoring guidelines (Gimsing *et al.*, 2019), Water Framework (2000/60/EC) and associated directives, QA/QC directive (2009/90/EC) and associated guidelines (e.g. SANTE/2020/12830). The typical approach taken when preparing regulatory submissions utilises readily available public monitoring data from online data repositories, digital maps and reports. Public monitoring data sourced from different regions/Member States are collected for: · different purposes [e.g. incident investigation, surveillance, compliance assessment], · using different sampling strategies [e.g. flow proportional/grab, seasonal/monthly], · by different organisations [e.g. water company, environment agency] using different sampling protocols, · analysed to different standards [e.g. ISO17025] by different organisations/methods, · and reported differently [e.g. LOD or LOQ reported; data quality (un)reported]. These public monitoring data provide valuable insight into the state of the environment, emerging issues and possible impacts, and are therefore in principle good data sources to fulfil the regulatory requirement. However, caution is required when collating, analysing and interpreting these datasets. Extensive data exploration should be undertaken such that all aspects of the dataset characteristics are fully considered for the assessment of the data. This poster outlines key issues in this context, relating to (i) data quality and harmonisation, (ii) data analysis e.g. dealing with outliers and non-detects, (iii) selection of robust science-based thresholds and (iv) derivation of relevant exposure values for compliance/impact assessment. It also considers interpretation of the results, confounding factors, and key aspects of conclusions on management and mitigation measures, e.g. ensuring that relevant uses, sources and pathway are addressed. The use of public monitoring data for local issue detection and for definition of local measures and stewardship programmes is also discussed.

3.08.P-Th054 Sorption and Desorption of Metribuzin in Soils Amended With Sugarcane Straw Biochar Produced at Different Pyrolysis Temperatures

Kamila Mielke, Kassio Mendes, Maura Gabriela Brochado, Alessandro Lima, Mateus Silva and Rafael D'Angeri, Federal University of Viçosa, Brazil

Biochar is defined as a carbon-rich product that can be used as a source of nutrients, soil carbon sequestration, waste management, and energy production. It has a high sorptive capacity and is a potential sorbent for organic contaminants in soil and water. Residual herbicides applied directly to the soil, such as metribuzin, may have their bioavailability, leaching and agronomic efficacy reduced due to the increased sorption provided by soil amendment with biochar. The pyrolysis temperature factor has shown to directly interfere in the sorption and desorption of the herbicide in the modified soil; however, the results may vary with the type of raw material, the soil characteristics and the herbicide analyzed. Therefore, the objective of the study was to investigate the interference of the sugarcane straw biochar produced at different pyrolysis temperatures on the sorption and desorption of metribuzin in soil. The experimental design was entirely randomized composed with three pyrolysis temperature of biochar (BC350, BC550 and BC750°C) added to the soil at application rates 10% w w⁻¹. The sorption and desorption study were carried out in an equilibrium batch method with five concentrations of metribuzin (0.5, 1, 2, 4 and 8mg L⁻¹) where the average

concentration corresponds to the highest recommended dose of the herbicide (1920g a.i. ha⁻¹) for sugarcane cultivation. The analysis of the herbicide was performed in High Performance Liquid Chromatography (HPLC). The sorption and desorption coefficient data were fitted to the Freundlich isotherm model (K_f). Sorption and desorption of metribuzin were 1.42 and 0.78mg (1^{2/1/n}) L^{1/n} Kg⁻¹, respectively, in soil unamended. BC750°C increased the K_f value of sorption of metribuzin 11-fold and decreased the K_f of desorption 45-fold compared to unamended soil. However, for the BC350 and BC550°C, the K_f value of sorption increased 4- and 6-fold and decreased the K_f for desorption 18- and 35-fold, respectively, relative to the unamended soil. The percentage sorbed was 63.8, 75.5 and 89.4% and the desorbed was 8.3, 5.8 and 3.7% to BC350, BC550 and BC750°C, respectively, relative to the initially applied herbicide. A pyrolysis temperature of 750°C produced a sugarcane straw biochar with higher sorption potential of metribuzin, which can become an alternative for immobilization of metribuzin in soil. The high sorption and low desorption of herbicide can interfere with bioavailability in soil solution and influence weed control.

3.08.P-Th055 Degradation and Sorption of the Herbicide Pelargonic Acid in Agricultural Topsoils and in Subsoils Below Railway Tracks

Thomas Poiger, Joanna Müller, Julian Angst, Roy Kasteel and Ignaz Buerge, Agroscope, Switzerland

Herbicides based on pelargonic acid are becoming increasingly important in organic farming but also for weed control in sensitive areas where use of synthetic organic chemicals may be less acceptable. In recent years, pelargonic acid also gained interest for weed control on railway tracks, preferably in combination with the herbicidal active substance flazasulfuron. While data required to estimate potential leaching of pelargonic acid from treated agricultural fields to groundwater (i.e. on soil degradation and adsorption) has recently become available, such data are missing for proper assessment of potential leaching from use on railway tracks. Therefore, we conducted degradation and adsorption experiments with various subsoils sampled at railway tracks. For comparison, we also performed such experiments with a number of agricultural topsoils. Our experiments confirmed that dissipation of pelargonic acid in topsoils is very rapid with half-lives of a few hours, when applied to soils at concentrations corresponding to typical agricultural application rates. In subsoils from railway tracks, degradation was 3-10 times slower than in topsoils, but half-lives were still < 1 day. We also conducted experiments at different starting concentrations. These experiments showed that degradation of pelargonic acid is strongly concentration dependent. At high starting concentrations, degradation initially is slower and becomes more rapid over the course of the experiment. Adsorption of pelargonic acid to subsoils was weak with Freundlich adsorption constants (K_f) of 0.06 - 0.31 mL/g. However, when normalized to the organic carbon content of the soils, the K_{foc} values fitted well within the range of 11 topsoils selected to cover a wide range of soil properties, particularly pH. Adsorption of pelargonic acid is pH-dependent and the sigmoidal curve fitted to the K_{foc} vs. pH data indicated an apparent pKa close to the pKa values reported in the literature. Overall, our data indicates minimal risk of leaching of pelargonic acid to groundwater, not only from agricultural but also from railway use of pelargonic acid.

3.08.P-Th056 Losses of Plant Protection Products Via Drainage Over One Growing Season on a Potato Field

Simon Mangold, Nora Bartolomé Gutiérrez, Isabel Hilber and Thomas Bucheli, Agroscope, Switzerland

On a 0.2 ha sized and drained field, we grew potatoes and applied plant protection products (PPP) according to good agricultural practice. In total, 20 different PPP were applied during ten application events in the growing period from April to August. In the rather dry year (431 mm in comparison to 517 mm precipitation in the longtime mean for this time span) precipitation caused eight discharge events in the drainage. An autosampler with discharge monitoring allowed flow proportional sampling and discharge peaks could be sampled with high temporal resolution of down to 30 minutes. Over the entire season 151 samples were collected and frozen until analysis and were measured with large volume injection liquid chromatography coupled to tandem mass spectrometry with electrospray ionization (LVI-LC-ESI-MS/MS). Almost all applied and measured PPP could be detected in the water samples. However, concentrations and duration of their occurrence varied widely. For instance, the apolar trifloxystrobin was detected only immediately after application, while the more polar metribuzin, once applied, appeared over the whole cultivation period. Together with the water discharge and the known amount of applied PPP, the exported fraction was calculated. The span of the exported fractions ranged from almost zero for the apolar compounds up to more than 0.4% for metribuzin, the compound with the lowest organic carbon normalized soil-water distribution coefficient (K_{oc}) in our substance selection. Total exported loads and maximum concentrations from the field experiment were compared with such predicted by the EXPOSIT model, which is used in the registration process of PPP in Switzerland to predict PPP concentrations and toxicological risks in surface waters. Measured emitted loads were in the range of the predicted values. However, peak concentrations in the field were much higher than the predicted ones, although the model is supposed to represent a worst case scenario. The 24h averaged concentrations (according to the duration of the discharge event in the model calculation) in the water samples show already up to 17 times higher concentrations compared to the model values. This discrepancy gets even bigger (factor of 60) if predicted values are compared with real maximum concentrations during a discharge peak (sampling frequency ~30 minutes).

3.08.P-Th057 Aging Reduces Bioaccessibility and Explains Persistence of Triazole-Fungicides Accumulated in Agricultural Topsoil

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We investigated the fate of triazole fungicides in the plough-layer of an agricultural field that is managed by the Danish Pesticide Leaching Assessment Program. The field has a detailed record of spray-applications. Topsoil was sampled from the field at regular intervals for more than two years starting in 2019. Surprisingly, we detected tebuconazole, propiconazole and epoxiconazole in the soil 10 to 20 years after the last spray applications. They furthermore showed almost constant concentrations

over time during the two years sampling. Metconazole, in contrast, was applied twice in 2019 within the sampling period. Metconazole was not detected before spraying, but after spraying, soil concentrations peaked and then declined rather quickly, levelling off at a constant concentration instead of approaching zero. We determined the bioaccessible concentrations to investigate the mechanisms behind the observed triazole-fungicide concentrations. Bioaccessibility was estimated by extraction with the complexing agent hydroxypropyl-beta-cyclodextrin (HPCD) that mechanistically mimics the microbial uptake of hydrophobic compounds. Tebuconazole, propiconazole and epoxiconazole showed low bioaccessibility, whereas metconazole initially showed high bioaccessibility. Aging of triazole fungicides was also demonstrated in a lab-experiment where soil from three organic fields was spiked with triazole fungicides followed by incubation for up to 2 years under oxic conditions. Subsamples were analysed by sequential extraction of the concentrations that were either immediately bioavailable (CaCl₂ extraction), bioaccessible over time (HPCD-extraction) or non-bioaccessible (hard extraction). The lab-experiment confirmed the field observations. Due to strong sorption and low degradability, a fraction of applied triazole-fungicides may persist in the topsoil and may accumulate after repeated application. This persistent fraction may slowly “bleed” 1,2,4-triazole to the groundwater many years after spraying.

3.08.P-Th058 Global Sensitivity Analysis for a MACRO Meta-Model for Swedish Drinking Water Abstraction Zones *Stefan Reichenberger¹, Thorsten Pohler², Sebastian Multsch², Nicholas Jarvis³ and Mikaela Gönczi⁴, (1)knoell France SAS, Lyon, France, (2)knoell Germany GmbH, Germany, (3)Swedish University of Agricultural Sciences, Sweden, (4)Swedish University of Agricultural Sciences (SLU), Sweden*

In Sweden farmers are legally obliged to apply for permits for pesticide use if their land lies within a drinking water abstraction zone. The standalone modelling tool MACRO-DB 4 developed by the Swedish University of Agricultural Sciences (SLU) is available for risk assessment and decision support. MACRO-DB 4 is used by local authorities, farmers/landowners and consultants, and is based on the well-established leaching model MACRO 5.2. However, the software is costly to maintain and slow for end users. Hence, a robust meta-model of MACRO-DB 4 was developed and integrated in a web-based tool (MACRO DB Steg2 v.5) that is fast, easy to maintain, and easy to understand for stakeholders. The meta-model (implemented as an R package) is based on i) a large number of MACRO simulations for the whole agriculturally relevant area of Sweden, and ii) a trilinear interpolation tool. The simulations comprised 18 climates, 72 soils, 1 typical crop (spring cereals), 3 application seasons (spring, summer and autumn), and 150 dummy compounds consisting of a grid of normalized Freundlich coefficient Koc, degradation half-life at reference conditions DT50 and Freundlich exponent. Target variables were i) the mean leaching flux concentration over 20 years at 2 m depth (PEC_{gw}), and ii) the 20-year mean concentration in large surface water bodies (PEC_{sw}; based on pesticide inputs via drainage and baseflow). The meta-model performs a trilinear interpolation (in the three-dimensional space of Koc, DT50 and Freundlich exponent) for log₁₀ of PEC_{gw} or PEC_{sw}, respectively. Different crops are taken into account in the web-based tool by adjusting the pesticide interception fraction according to the BBCH stage of the crop to be modelled at the time of application. In order to identify the most important input factors for PEC_{gw} and PEC_{sw}, a variance-based Global Sensitivity Analysis (GSA) was performed for the MACRO meta-model using the Sobol’ method. This method allows to i) identify first-order (direct) and higher-order (interaction) effects for each input factor (climate, soil, application season, Koc, DT50 and Freundlich exponent), and ii) rank the input factors according to their importance.

3.08.P-Th059 Agrochemical Exposure in Sri Lankan Inland Water Systems

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Sri Lanka has undergone a number of development efforts over the last few decades, including the development of a series of water reservoirs in the central dry zone resulting in the expansion of primarily rice growing agricultural communities. These rapidly developed communities were built with shallow wells that are impacted by surface water runoff composition, which may intermittently contain significant amounts of agrochemicals as they are applied onto the fields. Chronic kidney disease of unknown etiology (CKDu) has been a growing threat in tropical low-land agricultural regions globally, including Sri Lanka, and agrochemical pollution is suspected as one of the factors in this disease. This study compares water quality parameters including target agrochemicals in a transect along the major Mahaweli River and from wells in the local rice farming community of Wilgamuwa in the dry zone. Simultaneous health data was collected from CKDu patients who consumed water from these wells. This study shows elevated phosphate levels, with an average phosphate concentration of 0.47 mg/L in Wilgamuwa wells compared to the US EPA water quality criteria of 0.05 mg/L. This study also shows 68% of sampled Wilgamuwa wells and 20% of Mahaweli water samples had agrochemicals above water quality guidelines. While no direct relationships could be drawn between CKDu progression and water quality, these results indicate concern of both Mahaweli water quality and localized water contamination sources, and may help inform other studies of agrochemical exposure and CKDu globally.

3.08.P-Th060 Transport of WATER and Pesticides Through Sloping Vegetated Filter Strips on Macroporous Soil

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Vegetated filter strips (VFS) are well-known mitigation measures applied to reduce particle transport, soil, nutrient, and pesticide loss from agricultural fields. Transport of pesticides through vegetated filter strips have not been studied much under Norwegian conditions though, hence a field study was initiated in 2020 to generate more knowledge about the transport of water and

pesticides through vegetated filter strips and to generate data for model calibration. Two field runoff simulation experiments were performed to investigate surface runoff of water spiked with pesticides and bromide through sloping (9-14 %) vegetated filter strips (well established grass) and a plot with bare soil. The two trials were performed on strips of 3x6 and 1x3 meters respectively. Approximately 1000 litres of water were applied at the top of each plot and the runoff rate was set to 3 L/min in both trials. Very little surface runoff was generated as most of the applied water quickly infiltrated and leached out of the soil, applying to both trials. Subsurface flow along denser subsurface soil layers, through macropores and/or down to drainpipes dominated the transport patterns. In the first trial the water sampled showed very high concentrations of pesticides even after passing through the subsurface of 6-meter-wide vegetated buffer strips. Measured concentrations were as high as 42 and 74 % of the applied concentrations for the most sorbing (benzovindiflupyr) and the most mobile pesticide respectively (clopyralid). In the second trial 40 % of the amount of applied water was sampled and all about 300 samples will be analysed for bromide and pesticides. Here we present results from these trials and discuss factors that affect the transport of water and pesticides as surface runoff and/or by leaching in vegetated filter strips in sloping terrain, based on the results from two field-scale experiments in a loam soil.

3.08.P-Th061 Development of a Surface- and Groundwater Modelling Risk Assessment Tool for Predicting Exposure From Pesticides Used in Major Crops in Norway

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The risk assessment tool WISPE (The World Integrated System for Pesticide Exposure) was developed which includes the environmental fate and transport models winPRZM and EXAMS. WISPE is a computer modelling tool developed to evaluate the potential impact of crop protection products on the environment in Norway. WISPE makes it possible to estimate pesticide exposure in surface- and groundwater resources considering Norwegian conditions for 11 different crops. Originally developed in 2013, WISPE was updated in 2021 to be more flexible with adding scenarios, outputs, weather files and to utilize the most recent enhancements to winPRZM. This version of WISPE also includes two northern FOCUS groundwater scenarios. This poster presents the new user input interface and shows examples of the results.

3.08.P-Th062 Evaluation of the Effect of Band Application on Groundwater Concentration Using Two-Dimensional Leaching Modelling

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The leaching of agro-chemical substances in soil to groundwater for regulatory purpose in the EU and other regions is usually assessed by using 1-D (one-dimensional) models which exclusively considers vertical transport. One-dimensional modelling can be considered sufficient and appropriate for homogeneous field application (e.g. surface spray treatment to whole fields) since for this application type lateral transport and mixing effects are of minor importance. However, with the adaptation of innovative application techniques, such as precision farming including band and spot application, it is unclear if partial area application leads to locally higher ground water concentration under the applicated bands or spots or if lateral transport and dispersion processes compensate for the partial area treatment which results in a homogenous transport after a certain transport depth after complete lateral mixing. Two-dimensional (2-D) spatial modelling is used to investigate the influences of lateral mixing on the solute transport after partial area application. Different aspects are considered such as size of the applicated bands or spots, influence of distances between the bands or spots as well as influence of transport distance and lateral dispersion length. The 2-D modelling is performed with the HYDRUS-2D model using implementations of the FOCUS scenarios in HYDRUS 2-D as presented in Diamantopoulos et al 2017 (doi:10.2136/vzj2017.04.0070). Conclusions will be presented for which situations 1-D modelling can cover partial area treatment and under which circumstances 2-D modelling is necessary to consider the additional effects of band or spot application on the predicted concentrations in groundwater.

3.08.P-Th063 Transcriptomic Points of Departure Calculated From Rainbow Trout Gill, Liver, and Gut Cell Lines Exposed to Fluoxetine and Methylmercury

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There is great interest in the development, validation, and application of resource-efficient new approach methods (NAMs) in toxicity testing (e.g., in vitro models; toxicogenomics data). To this end, in vivo rodent and aquatic studies have documented that transcriptomics data may be analyzed using the benchmark dose-response (BMD) method. While the combination of BMD analysis and transcriptomics data has been used for in vitro studies on human cells, this approach has yet to be explored for in vitro studies with ecological species. The OECD standardized test #249 for cytotoxicity in gill cells from rainbow trout (RT), a key test organism used in ecological risk assessment, has garnered much attention as a NAM. Thus, the objective here was to establish an in vitro, transcriptomic-based BMD assay using RT gill, liver, and gut cell lines. Cells were exposed to fluoxetine (Flx; 0–1000 ppb) or methylmercury chloride (MeHg; 0–12,500 ppb) for the cytotoxicity assay, and subsequently with Flx (0–1000 ppb) or MeHg (0–250 ppb) for RNA Sequencing. Cell viability decreased to 78.1% in liver cells at 1000 ppb Flx, and < 24% across all three cell lines at > 2,500 ppb MeHg. The MeHg LC50 values for gill, liver, and gut cells were 301 ppb, 840 ppb, and 328 ppb, respectively. There were no differentially expressed genes (DEGs) in RT cells exposed to Flx, hence BMD analysis was not possible. The MeHg exposures resulted in 5,376, 3,714, and 7,236 DEGs, the maximum 1st peak of the BMD density plot was 14.46, 20.47, and 17.75 ppb, and pathway-level BMD analysis identified 16,8, and 21 enriched pathways in the gill, liver, and gut cells, respectively. No pathway overlapped across all three cell lines, however, ferroptosis, phagosome, and PI3K-Akt signaling pathway were common across gill and the liver, while apoptosis, peroxisome, and Fanconi anemia pathway were common across gill and gut. Overall, the liver was least sensitive, whereas the gill was marginally more sensitive in terms of cytotoxicity and the

gut was marginally more sensitive in terms of gene- and pathway-level BMDs. This study shows that several NAM concepts (i.e., in vitro, toxicogenomics, transcriptomics BMD) can be pulled into a single study design and can yield valuable dose-response information on both cell viability and molecular responses. Moving forward, there is a need to scale up this approach to test a diverse range of chemicals to determine if the information gathered is in line with expectations.

3.08.P-Th064 Developing a Data Table Tool for Farmers to Assess the Leaching Potential of Pesticides Under Different Soil and Climate Conditions in Norway

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Farmers in Norway have very few tools to assess the potential for leaching of the pesticides they apply to their fields. Some pesticides come with a general warning on the label stating that they may be prone to leaching and that caution must be taken. For farmers it is difficult to know when and how to follow this warning. In this project we wanted to make a tool where farmers can look up and identify the degree of leaching potential of all registered plant protection products for their crop under the soil and climatic conditions relevant for the field or area in question. In cases there are several alternatives, the idea then is that the farmer can choose the product with lowest leaching potential. In this project we identified the biggest agricultural regions in Norway and the dominating climatic and soil conditions in these regions and simulated the leaching potential of all approved plant protection products in the biggest crops (cereals and potatoes) in Norway using the groundwater leaching model MACRO-DB. Here we present the development of the table tool and some of the results generated in this project.

3.08.P-Th065 Harmonised Framework for Spatially Distributed Leaching Modelling of Pesticides Initiative: A 2022 Update

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Spatially distributed leaching modelling (SDLM) of pesticides is a methodology to estimate leaching potential over a large spatial extent such as national or European level. SDLM can help setting groundwater monitoring programs in context. It is described in the FOCUS groundwater report and foreseen to be used as higher tier leaching risk assessment as well as supporting monitoring studies. SDLM is already used as a higher tier assessment in the national authorization procedure in some EU countries and will probably become more important in future. At the SETAC Europe 2020 online meeting, the initiative was officially formalised as a SETAC working group, consisting of a triad of members from regulatory agencies, academia, and industries. A steering committee manages the effort to develop harmonized guidelines for spatial distributed leaching modelling across Europe and published a problem definition document describing the aim and scope of the work. In 2021 subgroups for Geodata and Modelling were established with each a specific focus. The Geodata subgroup is evaluating datasets that can be used to generate a spatial modelling scheme and associated scenarios. Data reviewed fall in several established INSPIRE metadata categories such as Agricultural, Meteorological, Land Cover, Hydrography, Soil, and many other categories. All data are evaluated for spatial coverage, resolution, temporal aspects, period covered, version control, accuracy, and frequency of updates. Data availability is assessed as well to ensure that all SDLM stakeholders can use the same datasets when developing their SDLM framework. The Geodata group primarily focusses on pan-European datasets that cover the EU27 and the UK. The modelling subgroup is evaluating the models to be used in the SDLM context. Specific attention is paid to runoff processes and the interaction with substance transport to groundwater. Pesticide leaching models developed for regulatory purposes do not consider these processes. In a spatial context, ignorance of runoff may lead to unrealistic leaching patterns, so options were evaluated how to include runoff in a harmonised way into the models (e.g., using the runoff curve number approach). The modelling subgroup is further investigating how other lateral loss processes, such as drainage, can be included. As the SDLM teams continue to work, this presentation provides an update to interested parties.

3.08.P-Th066 Spatial Variability of POPs in Air at European Background Sites

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The atmosphere is an important pathway for environmental transport of persistent organic pollutants (POPs) across national boundaries and into remote areas. While a limited number of atmospheric monitoring stations within Europe report concentrations of POPs in background air using active air sampling techniques, data on the spatial distribution of POPs across Europe remains limited. This limits our understanding of the main sources controlling the atmospheric burdens within Europe. The key objective of this study was to measure the spatial variability of concentrations of POPs in background air across Europe, and to use observations and models in concert to assess if the measured concentrations are mainly governed by secondary emissions or continuing primary emissions. In our study, polyurethane foam based passive air samples were collected at 101 sampling sites across 33 countries along a European-Arctic transect (35 °N to 81 °N, 51°W to 47 °E) during summer 2016. The samples were

analyzed for 31 polychlorinated biphenyls (PCBs) and 29 organochlorine pesticides (OCPs) by using a gas chromatograph coupled to a high resolution mass spectrometer. The Global EMEP Multi-media Modelling System (GLEMOS) was used to obtain further insight into source-receptor relationships of selected POPs. HCB and γ -HCH were detected in all samples and with the highest concentrations. PCBs, p,p'-DDE, Dieldrin and Endosulfan I were also detected frequently (>72%). Differences in spatial pattern were observed between the targeted POPs, e.g. γ -HCH was found to be highest at sites in eastern Europe whereas concentrations of γ -HCH were highest in western Europe. HCB was the only POP that was positively correlated to latitude, and also the only POP that had significantly increased over the last decade. The highest concentrations of PCBs were observed in central parts of Europe. Model predictions of PCB-153 by the GLEMOS model for each site suggest that secondary emissions are, on average, four times more important than primary emissions in controlling atmospheric burdens. The model predictions furthermore suggest that the relative importance of primary emissions are more influential in southern Europe compared to northern Europe. Our results illustrate the utility of combining observations and mechanistic modelling to provide insights on the relative importance of primary vs. secondary emissions for individual POPs, as well as on their main source regions.

3.08.P-Th067 Groundwater Modelling of Volatile Substances in the EU

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The modelling of volatile active substances leaching to groundwater and further refinement of these estimations can be complex, particularly when higher tier environmental fate studies are required either as inputs or to support a hypothesis on which a refinement relies. For active substances with indoor (e.g., permanent glasshouse) and open field uses, consideration must be given to the design and conduct of the higher tier studies to ensure they will be beneficial in the context of the current data package. This poster will look at groundwater models: comparing the input assumptions and potential for experimental refinement of these; the limitations of the models and the potential for higher tier modelling options. It also aims to outline the current state of applicable guidance in this area for relevant formulation types and uses.

3.08.P-Th068 Seed Dressing - an Overlooked Source of Pesticides and Their Transformation Products in Groundwater?

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1,2,4-triazole is of emerging concern regarding groundwater contamination. In Denmark, 3.4% of the groundwater monitoring wells are polluted with concentrations exceeding the European threshold of 0.1 $\mu\text{g/L}$. Spray application of the most common triazole fungicides, propiconazole, tebuconazole, epoxiconazole and difenoconazole, has been restricted by the Danish EPA. However, seed dressing, i.e., coating of seeds with a thin layer of pesticide formulation, has not been considered a potential source for 1,2,4-triazole formation. In the present study, we carried out laboratory column experiments to determine the leaching potential of 1,2,4-triazole from commercial barley seeds, coated with the triazole fungicides tebuconazole and prothioconazole. In addition, we followed the release of difenoconazole in an agricultural field site, after sowing of difenoconazole coated winter wheat. The results show, that 1,2,4-triazole leaches from soil columns with barley seeds coated with the two fungicides. Both, tebuconazole and prothioconazole, added to the leaching of 1,2,4-triazole, which reached concentrations up to 0.8 $\mu\text{g/L}$. However, at the end of the experiment after 63 days, only 1% of the total fungicide was recovered as 1,2,4-triazole. Extraction of the remaining fraction of tebuconazole and prothioconazole revealed that the parent compounds are released into soil but are rather immobile and stay close to the sow depth. Similarly, difenoconazole concentrations in the agricultural field site increased shortly after sowing of coated seeds to 0.8 $\mu\text{g/kg}$, while it was below 0.1 $\mu\text{g/kg}$ prior. It is evident from our results that not only the spray applications but also seed dressing contributes to the leaching of 1,2,4-triazole. It is therefore advised to include seed dressing when assessing the leaching risk of pesticides and their transformation products.

3.08.P-Th069 OECD Round Robin Test to Evaluate a New Test Design to Determine Plant Root Uptake for Regulatory Environmental Fate Modelling

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In 2018, the the German Environment Agency (UBA) initiated an OECD project to establish a new test design for the determination of plant root uptake for environmental fate modelling. The intended outcome is an OECD test guideline. The key part of the validation process is a round robin test that aims to evaluate the intra- and inter-laboratory accuracy and reproducibility of the new test protocol. The test design was based on a protocol developed by stakeholders from academia, regulatory authorities and industry [1]. An OECD experts group recommended to modify the preliminary design with regard to several experimental parameters and suggested to test plants with different growth stages (14- and 28-days old plants), three test item concentrations (0.5, 1.0 and 2.0 $\mu\text{mol/L}$) and suggested to reduce the incubation period to 48 h. In 2020, a pretest was conducted in one laboratory to check whether the proposed changes are feasible. The pre-test was performed with oilseed rape and wheat. 14- and 28-days old plants were incubated for 48 h with the test items [¹⁴C]-1,2,4-triazole and [¹⁴C]-TFA (trifluoroacetic acid). The plant uptake factor (TSCF, Transpiration Stream Concentration Factor) varied mainly depending on the test item concentration and the age of the plants. Keeping these parameters constant yielded rather homogeneous TSCF values. Based on this outcome, the round robin test with 10 labs was planned and started in 2021. If the results of the validation process prove the reliability of the test design, the protocol will be proposed for implementation as a new OECD Test Guideline in 2022/23. [1] Lamshoef, M., Gao, Z., Ressler, H., Schriever, C., Sur R, Sweeney, P., Webb, S., Zillgens, B., Reitz, M., 2018. Evaluation of a novel test design to determine uptake of chemicals by plant roots. *Sci. Total Environ.* 613, 10-19.

3.08.P-Th070 Collation and Analysis of Regulatory Field Trial Data for the Validation and Refinement of Crop Development Dates Used in Pesticide Exposure Modelling and Risk Assessment

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Determining appropriate pesticide application windows is an important component of pesticide exposure modelling; increasingly, regulators are constraining these application windows using estimates of the timing of BBCH crop growth stages from AppDate. For example, the “repaired” version of the FOCUS SW models will have AppDate integrated directly into the software shell to remove “subjectivity” from the selection of application windows. The AppDate (Klein, 2012) software calculates consistent application dates for use in FOCUS SW and GW modelling with the purpose of aligning the product GAP being assessed with the fixed model crops described by the FOCUS models. The dates for major crop development stages, e.g., BBCH 10, captured in AppDate are based on various sources, differentiated for GW and SW, with linear interpolation used for BBCH growth stages between these. Given exposure assessments are a function of the application dates this software generates, it is important to understand how realistic and representative they are; however, there is currently no comprehensive, quality-controlled, readily available, pan-European crop phenology dataset available to undertake such an assessment or for use in regulatory risk assessment refinement. The crop protection industry holds large quality-controlled datasets of BBCH crop growth stages within their efficacy and residue trials datasets that span the required crop types as well as the agronomic and pedoclimatic diversity of Europe. A Crop Life Europe (CLE) project has sourced efficacy trial datasets from 7 members and created a harmonised crop development dataset that allows for the assessment and, where required, the justification of more realistic and location specific dates in regulatory risk assessment refinements. The data collection process has highlighted that residue trial datasets are not typically stored in a format that makes them easily combined with the efficacy trials datasets. The harmonised database spans two decades (2000-2020), comprises >250 crops (combinable, top/soft fruit, pasture/grass, ornamentals, vegetables and herbs amongst others) and covers 25 EU Member States, 3 EEA countries as well as Switzerland and the UK. This poster describes the database created in more detail, presents preliminary results for representative crops and outlines opportunities for the further development of exposure science to allow for more accurate and realistic risk assessment.

3.08.P-Th071 The Underground of the Crops - Representative Agricultural Soils in Brazil Through a GIS Based Approach

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Environmental fate assessment of plant protection products (PPPs) for regulatory purposes requires soil studies to characterize route and rate of degradation, sorption, and mobility. Considering the unfeasibility of performing such tests on all soil classes that occurs in a country, and the lack of a clear definition about required local soil classes and characteristics, it is necessary to choose those which are the most representatives of relevant agricultural areas. In Brazil, the representative soils suggested by the authorities dates from 1996 (Portaria Ibama nº 84, de 1996), which were selected based on its mineralogical, chemical, and physical characteristics, in order to cover the clay mineralogy, pH, texture, and organic matter variability of main Brazilian soils (verbal communication, no official document published). Since 1996, Brazil’s agricultural area has increased by 105% (27.2 Mha in 1996 to 55.7 Mha in 2020). Recently, advances on satellite images to identify agricultural areas and the availability of high-resolution soil maps, it has been possible to estimate the main soils in use for agriculture by using geoprocessing (GIS) tools. The objective of this study is to identify the most representative soil types under agricultural use in Brazil nowadays based on a GIS analysis. Using updated soil maps on the scale of 1:250.000 from the Brazilian National Program of Soils (Pronasolos) and the land use maps provided by the Mapbiomas Initiative, it will be possible to identify the main soil types currently used for agriculture and inquire about the spatial representativeness of these soils in the studies to characterize sorption, mobility, and degradation of PPPs for regulatory purpose in Brazil. Moreover, this database can be used in a near future to generate more representative scenarios for PPPs exposure assessment as well as to support the choice of the most appropriated soils to be used in studies required for PPPs registration in Brazil.

3.08.P-Th072 Know Your Drift Facts

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Drift from pesticide application is a (if not the) major route of exposure for non-target organisms (NTOs). But how much do we actually know about factors that determine the quantity of spray drift reaching vertical collectors in the off-field (i.e., the NTO of interest)? These factors are (1) application technique (nozzle, spray volume, pressure, formulation, etc.), (2) environmental factors, i.e., wind and T°-plus-humidity and (3) specific characteristics of the NTO. Modelling, wind tunnel, and field experiments will be presented giving insights into the effects of some of these factors. Some conclusions so far are listed below. · Nozzle design (i.e., initial droplet size distribution) has a surprisingly small effect on downwind droplet size reaching an NTO at a given location. However, it does affect how much of the spray reaches the NTO (e.g. for conventional hydraulic nozzles, reducing volume by switching to smaller nozzle sizes with lower flow rate, droplets become smaller and drift quantity increases). · The distance downwind of the NTO and the wind speed are the main factors influencing the size of the droplets impacting on the NTO (largest changes in droplet size are between 1 and 5 m downwind). · There can be a noticeable effect of changing application

volume on NTO exposures, but this correlation is complex and depends on the method of changing volume (*e.g.*, changing speed *v.* changing pressure), type of nozzle used, and distance between crop and nozzle. It is hardly possible to change volume without changing other parameters like nozzle size or sprayer speed. However, given the use of drift-reducing nozzles, the effect is small enough for ground deposits to be considered independent of volume; airborne spray is independent of volume down to 50 L/ha. We generally hypothesise that only small changes in drift depending on volume will occur if droplet size remains approximately constant. The quantity of spray captured by NTOs with vertical structures appears to be correlated with the quantity (mass flux) and quality (drop size spectra) of the airborne spray, as well as the characteristics of the NTO (*e.g.* size and structure) and wind speed. Thus, a spray drift model combined with some description of the collection efficiency of NTOs should be sufficient to predict the quantity of spray reaching NTOs. Models like SiMoD (SSAU) are suitable as shown by comparing model results to field data. SiMoD confirms that the initial droplet spectrum has little to no effect on the droplet size distribution reaching the NTO and that distance and wind speed are the main factors influencing the size of the droplets impacting on the NTO.

3.08.P-Th073 Lessons Learned: A Regulators Experience of Applying the OECD 106 Evaluators Checklist

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The OECD 106 checklist was developed by EU Member State experts in collaboration with ECPA and EFSA to provide supplementary information to aid consistency in the conduct, evaluation and reporting of batch sorption/desorption studies. The checklist is a tool for applicants and regulators to help identify where deviations from the OECD 106 guideline could lead to significant and systematic errors in fitted parameters. It is not intended to replace the quality checks outlined in the OECD 106 guideline. K_{foc} and $1/n$ are critical input parameters to FOCUS modelling for groundwater and surface water. The models are sensitive to these input parameters. For example, a 10 % change in $1/n$ can lead to a 100 % change in PEC_{GW} . Therefore, robust studies performed to standardised guidelines are needed to have confidence in the modelled predictions. HSE's Chemicals Regulation Division has been applying the OECD 106 checklist to pesticide active substance evaluations for several years. Presented here are the key lessons learned from a regulatory perspective. The soil adsorption coefficient is normally derived using the 'indirect method' based on solution depletion. However, decisions made regarding the study set-up often result in the study not fulfilling key parts of the checklist and not being relied upon during the active substance evaluation. For example, a soil:solution ratio greater than 1:1 is used in cases where % adsorption is low; a long equilibrium time is used for volatile substances meaning the mass balance is unacceptable; or mass balance is calculated incorrectly by measuring % radioactivity not the test item. Altering the study design to account for these issues could result in the more cost-effective indirect method study being accepted. However, where these issues cannot be resolved by altering the study design, the 'direct method', where both soil and aqueous phases are analysed, is recommended. A common error for studies using the direct method include not accounting for the liquid entrained in the soil pellet.

3.08.P-Th074 New Tools for Predicting Environmental Concentrations in Soil in Regulatory Context - an Impact Assessment

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In 2017 the European Food Safety Authority (EFSA) published a new guidance with four tiers for exposure assessment of pesticides in soil. Tier-1 and Tier-2 procedures can be calculated with the software tool PERSAM, which generates input for Tier-3A calculations which are performed with the PEARL and PELMO tools. The final tier, Tier-4 addresses post-registration monitoring. We present an impact assessment of these future requirements for calculations of predicted environmental concentrations (PEC) and the associated ecotoxicological regulatory acceptable concentrations for soil organisms. 56 chemicals (parent substances and metabolites) covering a broad range of environmental fate parameters and several different uses were considered. PEC values calculated with the new tools were at all tiers substantially higher than corresponding values calculated according to the current FOCUS approach. Soil bulk density was identified as a main driver of the concentration increase. Soil bulk densities at geographic locations selected by the PERSAM tool for the calculations appear to not necessarily represent locations relevant for agricultural practice. Foliar interception and simulated wash-off, determined by crop growth stage at time of application, were identified as other main drivers for the increase in concentrations. Exposure increases were more significant for uses with higher interception, due to larger impact of simulated foliar wash-off on the amount of substance reaching the soil. Obligatory model and scenario specific correction factors embedded in the framework contributed to an increase of PEC values over all compounds and uses. Risk assessments for soil organisms were performed using PEC values from FOCUS and EFSA Tier-3A calculations, respectively. The comparison showed a substantial change in overall pass/fail ratios. In future roughly seven times more higher tier risk assessments will be triggered than currently arise, including necessity for field studies. Further refinement options need to be developed and implemented to obtain a new, workable soil risk assessment scheme. Time needed for performing such studies shall be considered in the implementation plan of the new assessment scheme. Please refer to the poster of Schimera et al for further details. Practical experience with the new models revealed a highly inefficient workflow, which will increase future workload for evaluators and applicants. This must be considered for the implementation plan: some additional usability features would be necessary, including improved reporting, access and operation with a substance database and automation of calculations. We propose a stakeholder discussion to resolve practical concerns related to the new tools.

3.08.P-Th075 Comparing PNEC to Swedish Pesticide Monitoring Data - How Reliable Is the Registration Process?

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In the registration process for Plant Protection Products, one of the assessments of a substance is calculating PNEC (Predicted No Effect Concentration) based on laboratory studies and comparing to PEC (Predicted Environmental Concentration), to ensure no unacceptable effects on aquatic organisms. In this abstract, we used PNEC values from the latest EFSA Conclusion for each substance (June 2021) and compared with measured environmental concentrations from Swedish pesticide monitoring data. The aim was to evaluate how well the registration process prevent negative effects on the aquatic environment. The Swedish national pesticide monitoring started 2002 and is mainly carried out in four small agricultural catchments (>90% arable land; 8-16 km²; Boye *et al.* 2019). Surface water is sampled automatically May to October, 100 subsamples per week, giving weekly average concentrations per substance analysed. These concentrations (E_i in Equation 1.) was divided with PNEC ($PNEC_i$) and summarized per year for all analysed substances ($=n$), giving an annual pesticide toxicity index (PTI; Figure 1). The results show that of the approximately 150 substances analysed annually, the majority of substances found (78) contributed with < 0.1% to total PTI during 2002-2020 (they have not been categorized into “approved for use (2021)” or year of ended approval). The four substances with highest total PTI are imidacloprid and picoxystrobin (approval ended during 2016-2018 in Sweden) and metiocarb and metazachlor (approval ended 2009-2015). Of the substances approved for use (2021) diflufenican and MCPA contribute most to PTI, with diflufenican having the highest PTI the last few years whereas annual PTI for MCPA show a decline. We conclude that 1) PNEC values seldom are exceeded for most pesticides measured in Sweden, although 2) some substances regularly exceeds PNEC and can be seen as problematic, but, 3) most of these substances have not got a renewed approval. Equation 1. $PTI = \sum E_i / PNEC_i$ Figure 1. Pesticide toxicity index (PTI) calculated for surface water from four intensively farmed catchments in the Swedish monitoring program of pesticides. Substances with total PTI higher than 0.1% categorized according to approval in Sweden or year approval ended. A lower total PTI than 0.1% are uncategorized. In 2009 the analytical method improved with more substances and lower detection limits. Boye, K., Lindström, B., Boström, G. & Kreuger, J. 2019. Long-term data from the Swedish national environmental monitoring program of pesticides in surface waters. Journal of Environmental Quality. DOI:10.2134/jeq2019.02.0056.

3.08.P-Th076 Modelling the Impacts of Climate and Socio-Economic Changes on Pesticide Use and Fate

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Agricultural use of pesticides helps to control a range of pests and diseases that threaten crops and thereby avoid yield losses and improve the quality of the food produced. However, pesticides applied on agricultural fields dissipate with time. The export of pesticides and their transformation products after application from the agricultural fields threatens the water quality of aquatic systems in many world regions. Climate change is further expected to intensify pest pressures and potential pesticide use by affecting agriculture in many ways. Changing climatic conditions can increase pesticide leaching due to increased and frequent rainfall, higher degradation rates due to higher temperatures or soil moisture contents. The indirect effects are changes in land use, the timing of crop cultivation, selection of other crop types, new pests and changed pest behaviour, etc. Additionally, several socio-economic factors influence pesticide use at the farm and national level, including regulation and legislation, economy, technology and crop characteristics. In order to better understand the pesticide risk to surface waters in the future, it is our aim to understand the influence of both climate and socio-economic change on pesticide use and fate. To assess pesticides and their impacts on water bodies, a variety of catchment scale models are available. However, most modelling approaches solely concentrate on the total amount or concentration of pesticide exported from a catchment and do not necessarily analyse the future change of pesticide and transformation products. We propose an integrated modelling framework to answer the research questions: What are the current significant climate and socio-economic drivers influencing pesticide use and emissions? How can climate change influence pesticide and transformation products emission trends? How will socio-economic change influence pesticide emissions? The integrated modelling framework helps to include adapting agricultural production to climatic (e.g., temperature, precipitation) and socio-economic drivers (e.g., land use, crop type, pesticide regulation) and quantifying pesticide emissions with the Zin-AgriTRA pesticide fate model. The ZIN-AgriTra is a catchment scale reactive transport model which can simulate agrochemical and transformation products exported from agricultural catchments. We use the Eur-Agri-SSP scenarios that extend and enrich the basic Shared Socio-economic Pathways with a regional and sectoral component on agriculture to explain the socio-economic change and climate projections for Representative concentration pathways to adopt climate change scenarios. The integrated modelling framework links the future scenario results from independent, standalone models that present crop rotation, land use, pesticide regulation and climate to the pesticide fate model (Zin-AgriTRA). The framework is applied to an agricultural catchment in Burgenland, Austria, to quantify pesticide pollution under future climate and socio-economic change up to 2050.

3.08.P-Th077 Pesticide Transformation Product Occurrences in Surface Waters As Ground Water Pollution Risk Indicators in the Context of an Extended Residence Time Aquifer

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Luxembourgish groundwater aquifers have recently been impacted by high concentrations of several pesticide transformation products that led to regulatory issues for about 1/3 of the drinking water supplies (Luxembourg considers all transformation products to be relevant and applies the 100 ng/L legal threshold). The consequent application restrictions or complete bans of

certain pesticides triggered switches in compound uses in several cultures. A leaching risk analysis had been conducted to orient agricultural counselling on the least impacting parent compounds and transformation products. However, since the leaching simulations relied on literature environmental property data of the pesticides (like fraction of TP generated), there was some uncertainty on the true impact of the identified TPs. Residence times of groundwaters in the main aquifer spanning on average between 10 and 20 years (with proportional recovery times once these waters are contaminated), a faster validation approach was needed. The hypothesis was established that the interflow component in surface waters would be a good indicator to estimate the amount of transformation products available for groundwater leaching. In that perspective passive sampling campaigns were established on four river basins of distinct hydrogeology to quantify the masses of parent compounds and transformation products transported during an entire season. All the predicted transformation products were identified and their occurrence varied in amount and timing in the different catchments. This poster discusses the influences of spatial use variability and hydrological connectivity of agricultural source plots to the magnitude of the occurrences as well as its potential link to the leaching modelling and its parametrization

3.08.P-Th078 Interpretation of Public Surface Water Monitoring Datasets: Characterising Pollutant Sources and Their Exposure Pathways to Assess Their Relative Importance

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Public surface water monitoring datasets are increasingly used within the regulatory process either directly (e.g. registration requirement under plant protection products [PPP] directive 1107/2009/EC) or indirectly (e.g. pharmacovigilance initiatives under the veterinary medicines [VM] directive 2001/82/EC). This public monitoring data provides valuable insight into the state of the environment, emerging issues and possible impacts. However, caution is required when collating, analysing and interpreting these datasets, especially where active substances are registered under multiple directives and used in a wide range of sectors. Interpretation of the results, considering confounding factors and understanding what might be concluded from such datasets is key to ensuring that policy responses and mitigation measures are targeted at the correct sector, use, source, pathway and use-period. This poster collates observations from several insecticide investigations where such a multi-sectoral assessment of sources and pathways was conducted. The number of direct and indirect sources and pathways to surface water can be quite complex and the range of indirect/direct sources and their pathways to surface water are identified and described. Some uses in certain sectors are difficult to characterise as information on the ingredients/treatments used in the production of imported/domestic products are poorly documented and little or no data is often collected/available. One such sector is that of textiles where raw fibres, fabric and garments may have been treated with insecticides at different stages of production for different purposes in countries with different regulatory control and compliance. Potential gaps in source and pathway characterization, their impact on interpretation of surface water monitoring datasets and the requirement for and role of additional legislation to address these is discussed.

3.08.P-Th079 First Outcomes of Glyphosate and AMPA Pesticides in Surface and Groundwater Bodies of the Puglia Region (Italy)

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There is a growing need to study the chemical quality of water bodies concerning Plant Protection Products (PPPs). Particular attention is paid to Glyphosate and its metabolite AMPA. Glyphosate is the world's best-selling herbicide for weed control. Its toxicity to ecosystems and human health is widely discussed worldwide. The excessive use of this herbicide promotes continuous monitoring in environmental matrices, particularly in surface and groundwater bodies. With the Water Framework Directive, each Member State of the European Union has adopted a National Action Plan (NAP) to combat water pollution for the use of pesticides. In agreement with the Italian National Institute for the Protection of the Environment (ISPRA), in the Puglia region (Southern Italy), a monitoring program of the residues of PPPs in surface and groundwater bodies has been approved. This study presents the results related to the analytical activities on Glyphosate and Ampa, provided by the Monitoring Program for 2018. A total of 216 samples were analysed, 98 of which were taken from surface water bodies and 118 from groundwater ones. Their particular chemical-physical properties and consequent analytical difficulties led to developing a method for better retention and separation. A pre-column derivatisation with FMOC-Cl coupled to Ultra High Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS/MS) has been applied. In surface water bodies, Glyphosate was present at 39.5% of stations, and in 20.8%, it exceeded the regulatory limit (0.1 µg/L). The AMPA was revealed with a percentage of 39.5%, while in 36.4%, it exceeded the legal limit. The mean concentration of Glyphosate was 0.28 µg/l, with a maximum concentration of 2.2 µg/l detected in Cervaro river and a minimum value of 0.03 µg/l. For Ampa, the average quantity was 1.32 µg/L with a maximum of 9.72 µg/L observed in the same river and a minimum concentration of 0.025 µg/L. For groundwater bodies, Glyphosate was present in 14% of cases, and 1.7% exceeded the legal limit. For Ampa, the presence was 3%, while the 0.4% exceeded the legal limit. The overall results showed a diffuse presence of the two pesticides along the whole Region. In order to predict their damaging effects on the delicate balance of ecosystems, develop models of sustainable management of environmental resources, and provide more environmentally friendly solutions over time, continuous and updated monitoring campaigns is crucial.

3.08.P-Th080 Environmental Monitoring and Risk Assessment of Pesticides Applied in an Agricultural Watershed Dominated by Avocado Orchards: East-Michoacan, Mexico

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Worldwide the increase of pesticides is a serious environmental and human health concern, even more in developing countries, where the use and application of pesticides are not regulated. Mexico is the largest avocado producer in the world, and Michoacan

the main producer state, representing 70% of the total land planted in avocados in the country. In the last 10 years, eastern Michoacan has experienced an expansion and intensification of avocado monoculture, mainly at the expense of traditional crops, and to a lesser extent in priority conservation forests. Hence, there is a need to assess surface and groundwater pollution risks as well as health risks due to pesticides used in avocado monoculture. To address this need, we first inquire the agricultural practices through interviews applied to 30 local farmers, asking them about the use and frequency of pesticides application. Weather data was obtained from six stations around the study area, and soil data from official cartographic sources and by examining the physicochemical parameters of five soil profiles. We assessed the concentrations of 23 pesticides in water from samples collected in 15 sites during the dry and wet seasons. The analysis involved an extraction step using solid phase extraction (Oasis HLB cartridges) and pesticides identification/quantification by liquid chromatography coupled with mass spectrometry triple quadrupole (LC-MS/MS). The risks for the ecosystem and mobility of pesticides in the basin is being evaluated using the Pesticide Impact Rating Index (PIRI) and Hydrus-1D model. Results show that most farmers apply at least six main pesticides every month (glyphosate, benomyl, paraquat, chlorpyrifos, cypermethrin and imidacloprid), as a “preventive treatment”. The soil types are dominated by volcanic soils: andosols in the productive zone, and lithosols and vertisols at the basin outlet. The final analysis is under process and results are expected by January. The chemical concentrations will then be confronted to the survey. The results of field survey, of the index calculation and modelling will show the potential risks linked to pesticides use and mobility in soils, which will be useful to support the need of regulated agricultural practices, to prevent health issues and pollution of natural resources.

3.08.P-Th081 A Field Study Protocol for Collection of Human and Environmental Data on Pesticide Use in Europe and Argentina

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Current farm systems rely on the use of Plant Protection Products (PPP) to secure high yields and the quality of crops and agricultural products. However, PPP may have considerable impacts on human health and the environment. We prepared a study protocol to determine the occurrence and levels of PPP residues in humans, plants, animals and other non-target species for exposure modelling and impact assessment. This was linked to a cross-sectional study to compare conventional and organic farm systems across Europe. Environmental and biological samples were collected during the 2021 growing season at eleven case study sites: ten in Europe (covering a range of climate zones and crops), one in Argentina (to inform the impact of PPP on growing soybean, an important European protein-source in animal feed). We monitored PPP fate in environmental media (soil, water and air) and in the homes of farmers, and complemented it by the collection of samples from humans, farm animals (cow, goat, sheep and chicken), and other non-target species (earthworms, fish, aquatic and terrestrial macroinvertebrates, bats, and farm cats). Data of PPP residues in environmental and biological matrices will be used to estimate exposures by modelling. These exposure estimates together with health and toxicity data will be used to predict the impact of PPP use on environment, plant, animal and human health. The outcome of this study will be integrated with socio-economic information leading to an overall assessment used to identify transition pathways towards more sustainable plant protection and inform decision-makers, practitioners and other stakeholders regarding farming practices and land use policy.

3.08 Measuring, monitoring and modelling of pesticide fate and mitigation in a regulatory context (Poster Corner)

3.08.PC-We13 Landscape Level Modelling for Derivation of Dilution Factors at Drinking Water Abstraction Locations *Shanghai Li, Sebastian Gebler and Tom Schröder, BASF SE, Germany*

The exposure assessment of plant protection products (PPPs) at drinking water (DW) abstraction locations is of growing interest for authorities, water suppliers, industry, and other stake holders and hence particularly addressed in the EU regulatory framework (regulation 1107/2009). From a landscape level exposure perspective, the target is amongst others to derive dilution factors from edge-of-field surface water (SW) concentrations (PEC_{sw}) to a potential drinking water abstraction location at the catchment

outlet. This dilution factor depends on various variables, e.g. the agricultural use area, the hydrology, connectivity and other processes that determine the fate of a chemical substance in a catchment. Currently, no generic guidance is available on the derivation of drinking water abstraction concentrations for conducting exposure assessments in the EU. An exception is the national approach of the Netherlands DROPLET, a simplistic but very solid first Tier approach. However, the Dutch approach underlies worst-case assumptions, e.g. all agricultural land is connected and releases water instantaneously to a water body, further it only considers the topographical characteristics of the Netherlands. Our work explores the feasibility of using a step-wise EU approach to derive realistic dilution factors at drinking water abstraction locations which are potentially prone to high exposure of PPPs. This involves, first, identifying potentially vulnerable drinking water catchments using a GIS-based approach. This was presented at SETAC 2021 by Gebler et al. showing the identification of potentially vulnerable drinking water catchments based on high resolution upstream agricultural land use and surface water distribution within a catchment. Second, evaluation of the dilution factor of PPPs at drinking water abstraction points using the spatially distributed eco-hydrological model - SWAT (Soil and Water Assessment Tool). The SWAT tracer experiments, which considers all these different processes that determine the dilution factors in a holistic manner, evaluates the dilution factor of PPPs in a conservative and yet realistic manner. Future work includes identifying proper temporal scales, the impact of connectivity as well as application timing on the quantification of the dilution factor within drinking water catchments in the EU.

3.08.PC-We14 Deterministic Modelling of Effectiveness of Runoff Mitigation in the Netherlands at Field Scale

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Runoff from agricultural fields may affect surface water quality due to the presence pesticides. In areas densely populated with water courses, as common in the Netherlands, farmers need effective tools to manage runoff events. Emissions to surface water can be mitigated by reducing the number and size of the runoff events. The aim of the study was to quantify the effect of mitigation measures on runoff reduction, parameterized for typical Dutch landscapes, crop, soil, and weather conditions. Two measures were considered: micro-dams in ridged fields and an infiltration trench at the edge of the field. The Soil-Water-Atmosphere-Plant (SWAP) simulation model describes deterministically the hydrological processes (Richards equation combined with the Darcy-Buckingham equation) at field scale, including estimates of runoff. 30 Years of rainfall events were simulated for different soil-crop combinations with and without mitigation measures. All arable fields in the Netherlands were attributed to one of 57 standard situations. The simulated runoff volume was plotted against precipitation volume for each unique SWAP simulation run for the 30-year period. The plotted data were then used to determine two parameters in the rainfall-runoff relationship for the reference and for the mitigation situations. The optimized rainfall-runoff relationships were then used to determine the effectiveness as a function of the precipitation, applicable for any precipitation event. With the effectiveness function and the set of 57 standard situations the effect of the mitigation measures for all arable fields in the Netherlands can be determined individually. The results of the study can be used to advise farmers for which crops and for which fields the mitigation measures help to reduce runoff from their fields. The deterministic modelling of runoff is appropriate for river delta areas with relatively little slope and for areas with shallow groundwater levels and where measures are mostly taken at the field scale.

3.08.PC-We15 The Integrated Drift Management Sprayer As a Use-Case for the Digital Label Infrastructure

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Farming is moving rapidly towards digitalization, ranging from control systems (e.g., self-driving vehicles in the field), to mapping of yields with respect to cropping variables (e.g., pest pressure, seed and soil quality) and digitally enabled risk mitigation measures, and to the overall integration of all inputs and outputs into planning software to drive decisions and document them. These changes need to be considered in the registration of plant protection products and will have to include, for example, changing application techniques and digitally enabled risk mitigation measures implemented on the label of plant protection products. However, labels are already complicated and further label complexity is in no-one's interest. A running project at Bayer AG – the Integrated Drift Management sprayer [IDM] – demonstrates a solution to increasing label comprehension and compliance by implementing the right label recommendations for products applied to a crop using automated spray application. This helps manage the complexity of dealing with multiple spray drift risk assessment compartments for vulnerable off-target areas (e.g., a residential area downwind from a stream next to a cropped field). In addition, the IDM applies a very simple change in the decision process by taking into account the wind direction and applying the label requirements automatically in a customised way in the field *during spray operation*. The software and the sprayer prototype have been developed integrating the customised software into an Amazone 28 m towed sprayer and uses the FieldView Drive system (also being developed by Bayer / Climate Corp) for documentation. The first proof of concept (PoC) for this approach to in-field application of label requirements modified to consider local wind conditions has been successfully completed at the ARVALIS research station at Boigneville, France in 2021 and will have been demonstrated, live, to the French regulatory authorities (ANSES and the Ministries of Agriculture, Health, Environnement). Thus, we have demonstrated the possibility that, eventually, all aspects of the regulatory oversight of pesticide spray application can follow a digitalized, in situ or field-localised risk assessment and integrate customized risk mitigation in real-time for local field conditions. The IDM PoC is a stepping-stone towards a vision of a fully integrated digital label.

3.08.PC-We16 Using Degradation Kinetics to Improve Our Understanding of Substance Behaviour to Support Regulatory Assessments

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For over a decade, regulatory scientists across Europe have collaborated to develop and utilise a harmonised approach to consider the degradation of parent compounds and breakdown products. Currently the main purpose of kinetic assessment is to produce key regulatory endpoints (DT₅₀, DT₉₀, formation fraction) for the use in environmental risk assessments or for comparison against trigger values in hazard-based classification systems. At the same time, there has been an increasing requirement to consider other behaviour observed in laboratory studies (for example non-extractable residues and stereoisomerism). Approaches are developing to support the interpretation of OECD laboratory studies but can vary across the regulatory regimes for pesticides, biocides, and general chemical under REACH. Whilst the new approaches look promising the practical implementation of the schemes into standard laboratory testing programs remains uncertain. In the past decade, our collective applied knowledge and understanding of degradation kinetics in various regulatory regimes have increased and kinetic analysis procedures and associated easy-to-use tools have been improved to the extent that there is now an opportunity to extend the standard kinetic approach to consider other observed chemical behaviours (where mass measurements are taken). With adaptations to the conceptual model, valuable insights could be gained to behaviours other than dissipation and/or degradation in environmental matrices. This insight can provide evidence to support refinements to produce more realistic regulatory risk assessments or better inform decisions on substance persistence. Additionally, these developments may potentially negate the need to conduct much more complex laboratory methodologies or decision-making frameworks in some circumstances. Case studies considering issues around handling non-extractable residues and stereoisomers are presented alongside conceptual models illustrating how data sets from standard OECD simulation studies could be analysed to provide this extra information and how it may help inform future regulatory assessments.

3.08.PC-We17 Improving and Implementing Passive Sampling Techniques to Monitor Pesticides in Rivers

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Active sampling, e.g. with an autosampler, is usually the method of choice to monitor pesticides in water streams. However, it requires specific and costly infrastructure (security, electricity). Passive sampling can be a good alternative or a complementary approach to monitor pesticides in diverse locations. However, the hydrodynamic conditions influence polar passive sampling and thus, need to be taken into account to obtain time-weighted average concentration over the sampling period. The mass transfer coefficient of the water boundary layer (k_w) is a key parameter that is mainly dependant of the water velocity. Determining k_w for laboratory calibration and field exposure will improve the understanding of the uptake process for polar compounds and thus the reliability of the results obtained by passive sampling. This presentation will discuss the calibration of polar samplers and the field application of a dual device comprised of non-polar and polar samplers. Experiments were done in a 4-channel system at four different water velocities and two temperatures. In a preliminary experiment, alabaster plates were deployed in a home-made Chemcatcher housing to determine k_w based on the mass loss of CaSO₄. Silicone disks were spiked with performance reference compounds (PRC) and deployed in the same conditions as the alabaster plates. Preliminary experiments demonstrated that PRC-spiked silicones can be used to determine in-situ k_w . Then, the polar samplers (Chemcatcher) were calibrated in the channel system to determine the sampling rates (R_s) for various pesticides. A relationship was established between R_s and k_w , allowing the calculation of in-situ time-weighted average (twa) concentration of the contaminants in water streams. This method was implemented in the field (two rivers) to test its feasibility. To do this, the non-polar and the polar samplers were co-deployed in a dual device (two weeks exposure). The non-polar sampler allows determining in-situ k_w , and the polar sampler accumulates the analytes. The practical aspects of the method were approved in the field.

3.08.PC-We18 Factors Affecting Spring Pesticide Spray Operations in Central Sweden: Towards Refining the FOCUSsw D1 Scenario Pesticide Application Timing

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Determining appropriate pesticide application windows and dates of application is an important component of pesticide exposure modelling and can have a marked impact on the outcome of surface water (SW) risk assessments, especially where these windows are on the fringe of the drainflow period. The current FOCUSsw pesticide application timer (PAT), considers patterns of rainfall to define a realistic worst-case application date. In certain cases an application window recommended by AppDate v3.0, also lacks realism. In the real-world farmers have to consider a range of other climatic and soil condition factors when deciding the practicality to spray pesticides. For example, can they actually access the field and traffic their land with a sprayer on any given day. A review of scientific publications considering soil workability/trafficability identified a range of soil moisture as well as plastic limits of deformation that might contribute to constraining pesticide applications in the PAT to days where a sprayer could access agricultural land. Incorporation of soil moisture deficit and plastic deformation limits into the FOCUSsw D1 scenario PAT showed promise but lacked sufficient direct applicability having been defined for other soil series, regions or continents. The Swedish University of Agricultural Sciences have collected detailed field level information of field operations within their O18 research catchment for >2 decades. The soils/weather in this catchment are closely aligned with that of the D1 scenario offering a good opportunity to explore factors affecting farmer decision making regarding spray operations. This poster outlines the approach taken to modelling soil moisture using MACRO v5.2 for 3 representative soil profiles and 7 representative crops assigned to each field in each year such that a spatio-temporal database of pesticide application dates, daily weather conditions and soil moisture metrics could be compiled. Analysis of this database allowed for the definition of factors and associated thresholds that affect the trafficking of soils and application of pesticides in central Sweden. Opportunities for use in the current and "repaired" FOCUSsw D1 scenario are discussed.

3.09 Micro- and nanoplastics: Towards the harmonized analysis for monitoring, effect studies and risk assessment (Part I)

3.09.T-01 Using Biological Traits to Identify Organisms Most at Risk of Microplastic Ingestion

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With plastic contamination of the environment now considered a planetary boundary threat, it is pertinent for us to understand which ecosystems and organisms are most at risk so as to best conserve our environment for the future. With levels of marine plastic contamination predicted to increase 50 fold by the year 2100 the seabed is acknowledged as one of the areas of great concern as ninety-nine percent of ocean plastic is predicted to sink to the seabed. Benthic sampling for microplastic contamination is under-represented in the literature due to the cost and equipment required to sample the benthic realm across the globe and the difficulties experienced in processing these samples. Hence, an alternative approach is urgently required to assess the potential impact of microplastics on this ecosystem. Our understanding of the impact of plastic contamination in these seafloor environments is paramount in elucidating the threat microplastics play to marine ecosystems. Given the challenges of monitoring the benthos, a organism focussed framework to assess uptake and risk of uptake of microplastics might be more appropriate. Traits based approaches offer one such framework. Here we test the hypothesis that biological traits can be used to assess the risk to benthic species and habitats from microplastics pollution. Traits can help disentangle interspecies differences and instead allow the prediction of the effects microplastics may exert at an individual to ecosystem level. We hope identifying species at higher risk from ingesting or interacting with microplastics will in turn, enable the field to undertake more targeted laboratory exposures and environmental sampling programs to investigate “at risk” species and create evidence for change. We addressed this hypothesis using a global meta analysis based on 1471 primary research articles returning 74 articles for final analysis; identifying 285 benthic associated species and over 17000 individuals who have been recorded to ingest microplastic. We present to what extent species traits (and other factors) confer vulnerability to microplastic ingestion, demonstrate organismal selectivity of plastic particulates and identify species groups that require further investigation in the hope that this will provide a roadmap for further research.

3.09.T-02 Systematic Development of Extraction Methods for Quantitative Microplastic Analysis in Soil Using Metal-Doped Plastics

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Monitoring microplastics (MP) in soil is crucial, not only to assess current exposure but also to evaluate possible mitigation measures. However, there are no standard methods for analyzing MP in soil - a very heterogeneous matrix that includes various inorganic and organic components. The plethora of approaches currently suggested in literature typically follows the pattern of 1. sampling 2. extraction and 3. detection and chemical identification. Previous studies overlooked evaluating the efficiency of such extraction methods on the dependence of sample content. Therefore, we focus explicitly on validating extraction methods to isolate MP from complex soil matrices by systematically investigating specific subgroups (sand, silt, clay, non-lignified and lignified biomass). To improve sample throughput when assessing extraction procedure(s) for individual or multi-component systems, MP with a passive inorganic tracer (Indium, 0.2% w/w) were used. Due to the selectivity of the metal tracer, the recovery rate of MPs from spiked samples could be quantitatively assessed using ICP-MS. Extraction procedures examined here include approaches that have been published in the literature and were complemented with new and extended concepts, including biotechnological advances for the degradation of lignocellulosic biomass. This covers density and lipophilic separation (for sand/silt), filtration through stainless-steel filters (for clay particles), Fenton's reaction (for non-lignified biomass) and a novel radical-enzyme treatment using enzymes from an anaerobic fungus strain (*Neocallimastix frontalis*), isolated from feces of Gaemse (for lignified biomass). Based on the results of the individual subgroups, sample-specific extraction chains were designed for lab-produced and standardized soil mixtures. The overall goal was to select a suitable extraction chain based on defined characteristics of the soil sample in question. Finally, a Nile Red staining protocol was developed to assess how appropriate these extraction chains would work for non-metal-doped MPs, which requires a filter with minimal background residues for chemical identification using e.g. FTIR-spectroscopy. This allows a distinction between the number of residues in brightfield mode from MPs counted in fluorescence mode. These systematically developed methods thus open new perspectives on soil microplastic analysis and are a step towards further harmonization of extraction protocols in solid matrices.

3.09.T-03 Towards Reproducible Microplastic Quantification: Advanced Automation of Raman Microscopy Enhanced by Machine Learning

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Microplastic (MP) analysis is a complex, multi-step process that includes sampling, sample treatment, measurement and data analysis. For each step, a multitude of protocols has been developed, but mandatory standards do not yet exist. Thus, comparability of results is an issue, even if the same methods are used. Result deviations can occur at each point in the process, but to ensure precision and accuracy, it is necessary to evaluate the contribution of each step. Here, we investigate measurement and data analysis. For the measurement, we focus on a combination of optical particle detection and Raman microspectroscopy. We compare the MP quantification in riverine surface water (particle sizes > 50 µm) and aquatic reference samples (particle sizes

> 10 µm) obtained with the software tools ParticleScout, which is commercially available, and GEPARD, which is an open source program developed at the IPF Dresden. Both tools allow a highly automated analysis. ParticleScout as the proprietary software of the Raman microscope manufacturer WITec fully utilizes the instrument's capabilities, which results in optimized analysis speed, precision and ease of use. The GNU GPL-licensed GEPARD can be adapted to a broader range of instruments. Furthermore, it provides a unique annotation module for correcting inaccuracies in the particle detection. To illustrate the result variations that occur when measuring the same sample with different settings, we compare the results obtained by varying the software, the Raman parameters and the type of Raman instrument. For the analysis of the Raman spectra, a common method is an automated library search that uses comparison algorithms, such as the Pearson correlation coefficient, to assign the spectrum with the best match. This is sensitive to effects such as fluorescence or a poor signal to noise ratio. Especially single component library searches, which are suitable for fast analyses and highly automated workflows, may misidentify spectra that contain bands of more than one component. Hence, the result often requires a manual follow-up control, which is time-consuming and prone to introducing human bias. As an alternative, we present first results for a machine learning-based approach for the analysis of Raman spectra. In a proof of concept, we train a model to identify several of the most common polymer types and compare its performance to the routinely used spectra analysis on the same samples.

3.09.T-04 Evidence of Underestimation in Microplastic Research: A Meta-Analysis of Recovery Rate Studies

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Society is increasingly interested in the levels of microplastics in the environment and the possible associated risks. The success of the research is dependent on how accurate and reliable the methods are when isolating microplastics from an environmental matrix. However, complicated, multi-staged methods are now being developed at an increasingly fast rate to combat the discovery of microplastics in new complex media. As a way of validation, these new methods should be subject to a recovery rate study. This involves 'spiking' the study matrix with known amounts of microplastics, running the method in question and calculating the amount of microplastics recovered. However, an issue in the study of microplastics is that this type of method validation is rarely undertaken. Here, 71 recovery rate studies were pooled for a meta-analysis. Sediment was found to be the most-studied environmental medium and saline solutions were the most frequently used reagent to isolate the microplastics. Polyethylene and polystyrene were most commonly used as spiking polymers, which is representative to highest polluting microplastics in the environment. The highest recovery rates were found with plant material, excrement, and whole organisms (>88%) and lowest recoveries from soil, fishmeal and water (58-71%). We have provided what we believe to be the first (to our knowledge) indication for an approximate general underestimation of environmental microplastics of 14%, based on the studies we reviewed. Furthermore, to improve the current lack of standardisation in microplastic research we recommend improvements in the use and quality of reporting in recovery rate studies.

3.09.T-05 Production of Micro and Nanoplastic Reference Materials

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The validation of analytical methods for the identification and quantification of micro and nanoplastics in environmental matrices is hindered by a lack of standard reference materials (SRM). These materials are required for the harmonisation of analytical methods and the generation of comparable and reliable data. As a result, uncertainties associated with the measurements from individual laboratories can occur due to contamination, over-estimation, and under-estimation of nano and microplastics from environmental samples affecting the comparison and interpretation of analytical data from different laboratories. Until now, the Norwegian Institute for Water Research (NIVA) has focused on making reference materials (RMs) in the microscale, i.e., from 50 µm and up to 1 mm of the following polymer types: polyvinyl chloride (PVC), polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS), as well as polymer mixtures, in the form of effervescent soda tablets. These RMs have been used in inter-laboratory comparison studies worldwide as well as in microplastic recovery tests in pyrolysis gas chromatography mass spectrometry (Pyr-GC/MS). The RMs have been analysed by different laboratories using different techniques, including light microscopy, micro-Fourier transform infrared spectroscopy (µFTIR), Raman and Pyr-GC/MS. The next step in generating relevant RMs is to focus attention on the size fractions below < 50 µm. This is of importance as the detection of small particles will allow the generation of data relevant e.g. for the impact of nano and microplastics on biota. These materials will be generated by cryo milling and size fractionation and will be characterised by several techniques. The materials will be made available through the NORMAN network and the EU project EUROQCHARM.

3.09 Micro- and nanoplastics: Towards the harmonized analysis for monitoring, effect studies and risk assessment (Part II)

3.09.T-06 Raman Microspectroscopy-Based Analysis of Micro- and Nanoplastics: Ready for Harmonization and Standardization?

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Microplastics (MPs) and nanoplastics (NPLs) are found in the environment all around the globe as well as in drinking water and food, raising concerns about their impacts on the environment and human health. To adequately address these issues, reliable

information on their ambient concentrations is needed. Here, vibrational spectroscopy methods which allow for the chemical identification and quantification of plastic particles, providing information on the particle number, size/size distribution, and shape can be applied. The size range is determined by the detection limit of the method, i.e., 1 μm or even below (down to approx. 300 nm) for Raman microspectroscopy (RM). Thus, RM is suitable for the analysis of both MPs and (partially) NPLs. Since a very high number of MP particles has to be measured in order to achieve representative results, automation is necessary. Therefore, we introduced open source program *TUM-ParticleTyper* (doi.org/10.1371/journal.pone.02347), which enables the detection, quantification, and morphological characterization of fragments, including particles and fibers in images from optical microscopy. It can be used to randomly select targets for subsequent RM analysis of up to 7000 particles/fibers on the entire filter. For the analysis of fragments down to 1 μm , we propose to choose windows for the subsampling randomly, and to apply a bootstrap method to provide an error quantification with confidence intervals from the available window data (doi.org/10.1007/s00216-021-03326-3). To enable the representative analysis of NPLs, we developed the online coupling of field-flow fractionation (FFF) with RM (doi.org/10.1021/acs.analchem.9b05336). The FFF system is used for the separation of particles, while chemical identification is performed by RM. The online detection in flow cell is enabled by utilizing an optical tweezer-based particle retention. The online coupling has been realized for asymmetric flow FFF (AF4) and centrifugal FFF (CF3) and validated for the size range of 100 nm – 5 μm and for different polymer and inorganic materials. Thus, RM can be efficiently applied for the reliable and representative MP analysis in the entire size range (down to 1 μm). Here, not only harmonization (doi.org/10.1007/s00216-021-03498-y), but also standardization is in progress. The RM-based analysis of NPLs will, however, require further development and validation of the procedure, before the steps towards harmonization and standardization can follow.

3.09.T-08 Reproducible Analytical Pipelines (RAPs) in Environmental Plastics Analysis: First Outputs From the EU EUORqCHARM Project

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Plastic pollution has become a global environmental and societal concern in recent years. Numerous protocols have been developed to monitor plastic debris, but these are rarely comparable. This has hindered gathering of knowledge regarding pollution sources, development of monitoring programmes and risk assessments and implementation of mitigation measures. To develop long-term solutions to reduce plastic pollution, it is essential to establish methodologies that are harmonised, or at least interoperable. EUORqCHARM addresses this by critically reviewing state-of-the-art analytical methods. The number of articles published in the scientific literature about plastic monitoring methods has increased exponentially in recent decades and scientists face a huge amount of scientific literature when designing studies and monitoring programs about litter in the environment. Review articles are a solution to reduce this amount to a digestible summary. Traditional reviews based upon readers experience may lack rigorously defined criteria to identify and select the vast number of studies in an area and two different authors may reach different conclusions depending on the chosen articles. Systematic reviews are used to quickly and accurately identify scientific discoveries and to mitigate the biases often found in traditional review. A Systematic Review is a research summary that uses a structured, reproducible approach, often supplemented by a meta- analysis. It has been carried out and matrix- and size-adapted methods have been highlighted, which may eventually be incorporated into plastic monitoring programmes. Harmonisation for large scale monitoring requires flexibility, comparability and reliability, therefore Reproducible Analytical Pipelines (RAP) are proposed based upon Systematic Review data, resulting in a catalogue of RAP procedures for nano-, micro- and macro-plastics for sediments, water, biota and air target matrices. Each RAP is validated in terms of Technology Readiness Level and SWOT analysis.

3.09.T-09 Hierarchical Analysis for Microplastic Classification Applying Fourier Transform Infrared Imaging System

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Microplastics (MPs) are defined as microscopic plastic particles in the range of 1 μm - 5 mm. These particles are recognized as emerging pollutants that are broadly present in the environment. Although this emerging pollutant needs to be addressed, the field is still relatively young, and robust standardized analytical approaches for sampling, treatment, and analysis are still in development. This work aims to develop an analytical method for the classification of MPs using Fourier Transform Infrared imaging system (?-FTIR) and hierarchical analysis (HA). Principal Component Analysis (PCA), Soft Independent Modelling of Class Analogy (SIMCA) and Partial Least Squares Discriminant Analysis (PLS-DA) models were evaluated in order to retrieve the three major information of ?-FTIR imaging measurements (Cary 620 FTIR microscope from Agilent Technologies) for MP analysis: background (membrane), MPs (target information) and Natural Matter (interfering particles, which often occur despite sample processing). This strategy was evaluated to classify the most common polymers produced worldwide (PE, PET, PMMA, PVC, PC, PUR, PA, PS, PP, ABS and PBT). Statistic models assessment were realized using the misclassification error, specificity and sensibility for cross-validation and prediction steps. PCA successfully selected the region of interest by removing any pixels not related to the particles in the image. Both SIMCA and PLS-DA showed great average sensitivity ($S_n \approx 1$) and specificity ($S_p \approx 1$) for sorting natural matter and discriminating the polymers, respectively. As for misclassification error, an average of 3% and 0.2% were obtained for SIMCA and PLS-DA, respectively. The hyperspectral images were also analyzed to quantify particle abundance and size automatically. This approach was validated and could classify particles tested on a sample with a mixture of MPs spiked with natural matter (cellulose and protein of different kinds). The proposed method demonstrated a

fast and efficient automated approach for microplastic polymer characterization, abundance numeration and size distribution. Moreover, a more detailed description of the particle was provided using the spatial information of the image to determine the size of MP particles and their frequency. In addition, HA is faster than the traditional library searching method, with results obtained in seconds once the models have been developed, improving the MPs throughput analysis.

3.09.T-10 Microplastics in Waste Water Treatment Plants: Monthly Analysis With FTIR and Py-Gc/Ms and Methodological Improvements

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Microplastics (MP) were shown to be present in various environments around the world. In order to better understand distribution patterns and to provide a solid basis for risk assessments of this organic pollutant, comprehensive datasets on MP concentrations and chemical compositions are needed. This study focussed on the effluents of two German wastewater treatment plants (WWTP) and investigated the temporal input of MP into the receiving river systems by performing a year-long sampling campaign with monthly sampling events. MP item data (minimum size: 11 µm) were generated using Fourier Transform Infrared (FTIR) spectroscopy, under the application of an improved polymer database. The database adaptation allowed for an improved data quality, as it counteracted matrix interferences due to residual plant material on measurement filters. Beside item data, complementary MP mass data were gained by the application of pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) (for one WWTP). Both item and mass data showed homogeneous polymer compositions over the sampling year, generally dominated by polyolefins. Elevated MP item and mass concentrations occurred during winter months, and were accompanied by either heavy rainfall (resulting in increased discharge), total organic carbon or elevated turbidity values. These findings underline the necessity for the integration of background parameters in MP monitoring studies. Finally, by providing monthly data over one year on MP masses and items, this work contributes to the current knowledge on temporal MP dynamics in WWTP effluents, and can therefore be a useful baseline for future monitoring studies.

3.09 Micro- and nanoplastics: Towards the harmonized analysis for monitoring, effect studies and risk assessment (Part III)

3.09.T-11 Development of an Open-Source Hyperspectral Database and Model for Microplastics Classification and Comparison to Nile Red Staining

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Microplastics (MP) analysis is time-consuming, which limits our ability to monitor and respond to plastic pollution. Hyperspectral imaging is a rapid spectroscopic technique that can be used to image relatively larger surface areas than µFT-IR and Raman imaging systems. In this work, we develop a database and classification model for four polymer types (PP, PE, PET, PS) based on pristine, consumer product sourced and environmentally weathered plastics. A SIMCA chemometric model is used to classify hyperspectral images of spiked microplastic samples on glass fiber filters. Model performance parameters vary based on polymer type; however, specificity, sensitivity and accuracy are all greater than 85%. The quantification of MP sizes (area, Feret diameter, perimeter, circularity) is determined in parallel by HSI and Nile red staining with fluorescence microscopy to assess the performance of HSI. MP < 150 µm Feret diameter are not consistently detected by HSI when compared with results from Nile red staining; however, estimates for Feret diameter are consistent with Nile red staining for MP > 500 µm. HSI is shown to be a rapid method to accurately identify MP above 500 µm. With a simple user-friendly interface on an open-source application, this MP analysis workflow minimizes the need for expertise in spectroscopic methods enabling more researchers to rapidly carry out MP analysis. The workflow for analyzing hyperspectral images is open source and will be integrated into OpenSpecy, an existing database and analysis tool for MP spectral analysis. HSI could be a key technology for environmental monitoring frameworks where rapid imaging (< 30 seconds per sample) and analysis of multiple samples is required, however MP size ranges of interest must be above the limit of detection for the hyperspectral instrument being used.

3.09.T-12 Detection and Identification of Microplastics in Biota Using Nile Red and Machine Learning: Validation of an Innovative, Cost-Effective Approach

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Plastic pollution has become one of the most ubiquitous threats the oceans are facing nowadays. Microplastics (MPs) are of special concern as it has been shown these are ingested by a wide range of marine species from different trophic levels. However, the environmental implications of MPs ingestion, coupled to the toxicological relevance of different MP polymers and their chemical composition, remain poorly understood. This highlights the need for standardized, cost- and time-effective monitoring procedures to accurately and routinely determine the abundance, composition and distribution of MPs in the marine environment, to allow for effective management strategies. We developed an innovative approach for MP analysis in biota, thereby combining the advantages of both high-throughput screening and automation. We evaluated the method in mussel and different fish

gastrointestinal tracts (GIT) samples. The pretreatment involved a two-step digestion (10% KOH and 15% H₂O₂) at 50°C, with a stainless steel filtration step in between and an optional density separation step (NaI) to remove sediment, followed by filtration over a PTFE-filter and staining with the fluorescent dye Nile red (1 µg/ml acetone). MP detection and identification of the polymer types was done using two machine learning decision models. The first model predicted with high accuracy whether a particle was plastic or of natural origin. The second model allowed to identify the MP polymer type on the base of RGB colour data, which are extracted from Nile red-stained MPs, photographed through a fluorescence microscope under blue, green and UV filters. The efficiency and suitability of our approach was validated by spiking six MP types into the mussel and fish GITs, each one varying in the combination of polymer type (PAN, PET, PP, PS and PVC), size (250 – 1000 µm) and shape (particle/fibre). The validation took into account the accuracy, precision, limits of detection and quantification, selectivity, specificity, and robustness. This unique approach of high-throughput screening and machine learning automation, proved to be promising for the cost- and time-effective routine analysis of MPs in mussel and fish GITs in a simple, yet reliable way.

3.09.T-13 Characterization of Micro- and Nanoplastic Exposure in Human Tissues Using an Exposomic Framework

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Despite rapid advances in our understanding of micro- and nanoplastics (MNPs) in environmental matrices, significant data gaps hinder our understanding of MNP exposure in humans. Studies involving small sample sets investigating specific tissues have thus far established the presence of MNPs in human feces and placenta^{1,2}. Further work is required to more fully understand MNP exposure across a range of human tissues to deduce associated health outcomes. However, this goal is hindered by analytical challenges associated with the heterogeneous and complex nature of MNPs. Most environmental studies of MNPs focus on a few candidate polymers or chemicals that capture only a small fraction of MNP complexity. To overcome these and other limitations we have developed an untargeted HRMS analytical framework to characterize known and unknown MNPs, their monomers and additives, and related exposure biomarkers and biological response. This analytical framework combines 1) pyrolysis (GC) gas-chromatography with HRMS (Pyr-HRMS) to characterize MNP levels, 2) alkaline-assisted hydrolysis with liquid chromatography HRMS (AAH-HRMS) to measure particle monomers and additives, and 3) untargeted liquid chromatography with HRMS (LC-HRMS) to characterize the metabolome for the presence of MNP constituents and additives and biological response profiles. This framework was applied to placenta samples, virgin plastics, and weathered plastics to validate our approach. Pyr-HRMS detected a range of common MNP types in placenta tissue, including PS, PE and PMMA which were detected at a range of concentrations from 10-2650 ng/g. LC-HRMS revealed that free and particle-bound small molecule profiles were significantly different between virgin and weathered particles. Results to date validate the application of this framework to contemporaneously characterize polymers, free monomers, additives, and biomarkers of disease in human tissues; this framework provides an important toolset to expand our understanding of MNP exposure in humans and other biological receptors.

3.09.T-14 Quantification of NanoPlastics Association With Cells: Advances in Single-Cell Inductively Coupled Plasma Time of Flight Mass Spectrometry (sc-ICP-TOFMS) for Fast and Sensitive Detection of Metal Doped NanoPlastics

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Plastic pollution is a growing environmental issue and has triggered a need for appropriate hazard assessment strategies. Particulate plastic is present in the environment along a size continuum (macroplastics, microplastics and nanoplastics), and different size classes will dominate different fate and transport routes, as well as possibilities for biological interactions. Nano-sized plastics may pass through biological barriers and enter cells. Unfortunately, analytical tools for sensitive and quantitative detection of nano-sized plastics in cells and tissues are largely lacking. Indeed, the assessment of nanoplastics association with cells is more analytically challenging than other metal-based nanomaterials (e.g. engineered nanomaterials) considering that (nano)plastics chemical composition is more difficult to distinguish amidst high concentrations of organic matter with lower analytical detection limits. Therefore, assessing risks of (nano)plastic pollution demands creativity and requires innovative analytical methods, even in a laboratory context. An elegant approach is to dope nanoplastics with an inorganic tracer, thereby allowing for a more effective detection and tracking in complex matrices using common analytical techniques appropriate for metal analysis, such as ICP-MS. In this work, we synthesized nanoplastics particles with an embedded inorganic fingerprint (Pd) and used them to investigate the association of nanoplastics with human cells grown in culture using single-cell ICP-TOFMS. The simultaneous elemental detection of ICP-TOFMS enables the measurement of the full mass spectrum, thereby allowing the simultaneous detection of a cell elemental “fingerprint” together with the metal tag of the nanoplastics particles. Consequently, this method offers a direct quantitative measure of the nanoparticle-cell association. The potential for this approach is highlighted by analysing human cells (THP1 monocytes and A549 lung epithelial cells) exposed to low concentrations of Pd-doped nanoplastics (0, 5 and 50 mg/L; 24h). Additional challenges and improvements related to sample preparation, sample introduction and transport efficiency were also overcome to develop a robust and reliable method. Collectively, the study presents a strong foundation for a powerful analytical method to explore nanoplastics exposures in a variety of conditions, ranging from ecotoxicological studies to human health related questions.

3.09 Micro- and nanoplastics: Towards the harmonized analysis for monitoring, effect studies and risk assessment (Virtual Only)

3.09.V-01 Arctic Seabirds: Tools to Support Spatial, Temporal and Source Monitoring of Litter and Microplastics Across the Pan-Arctic

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Plastic pollution has been reported to affect birds in the Arctic over the last few decades. There are strengths and limitations to monitoring litter and microplastics using Arctic birds. Spatial and temporal trends of plastic pollution can be based on northern fulmars (*Fulmarus glacialis*) as an environmental indicator. Similarly, the use of gulls and skuas across the Arctic can be used to track sources of litter and microplastics in the region as they regurgitate pellets that include indigestible material that has been recently consumed. We also recommend the monitoring of plastic additives in consumed species and tissues, preferably coordinated with other contaminant monitoring, to inform questions related to human health. We recommend that future programs consider a range of monitoring objectives, and consider monitoring birds as part of the suite of tools for monitoring plastic chemical additives and effects, as well as understanding the source and surveillance of litter and microplastics. Local and Indigenous researchers and community representatives are critical to decision-making processes for priority species and research questions in their areas, and should be involved in every step of the research. An important consideration is the ethics of reporting and publishing contamination results for wild food species, even if the results are null or low, as Arctic birds are an important food for many communities across the Arctic region.

3.09.V-02 Assessment of Photocatalytic Degradation of Engineered Polystyrene Nanoplastics (PS-NPs) in the Aqueous Medium Using Titanium Dioxide (TiO₂) Nanoparticles

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Natural and engineered NPs are emerging pollutants that pose risks to human and ecosystem health due to their persistent nature and bioaccumulation potential. Traditional water and wastewater treatment methods may treat NPs; however, these methods cause secondary pollution by transferring the pollutants to other mediums in concentrated forms. Photocatalytic degradation using TiO₂ nanoparticles is a green method that treats organic pollutants in water by mineralization through direct oxidation and formation of free radicals. In this study, anatase form of TiO₂ (10-20 nm, 80 m²/gr) and a mixture of 80/20% anatase/rutile form of TiO₂ (18 nm, 60-90 m²/gr) nanoparticles were used for degradation of engineered PS-NPs with an average size of 22 nm as a model for NP pollution in water. Preliminary results indicated that the mixture of anatase/rutile form of TiO₂ had greater treatment efficiency resulting in up to 90% degradation and mineralization of PS-NPs under optimum operating parameters: 96 mg/L dose for TiO₂, water pH of 6.35, and irradiation time of 6 hours under UVC from 4.5 cm distance and at 300 rpm magnetic stirring. Initial results for potential reuse of TiO₂ nanoparticles indicated that they have high potential for reusability after five reuse cycles without recovery. High photocatalytic degradation efficiency for reuse cycles should be further investigated to be applied at commercial scale for treating NP pollution in water.

3.09.V-03 Finding Microplastics in Hyperspectral Images - a Guide Through Current Methods

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The identification of microplastics (MP) in vibrational spectra is challenging due to method- and sample specific variables and large datasets. Several automatization approaches have been proposed for analysis of vibrational spectra, making it an important topic in the ongoing discussion about harmonizing MP research. We here aim to provide a critical overview on the methods proposed and hint at remaining challenges [1]. A common method is to compare query spectra to a database of reference spectra. It has been shown that not only the selection of database entries but also of the metric for spectral similarity substantially influences the results. For instance, inclusion of weathered particle spectra is highly recommended [2]. Both supervised and unsupervised machine learning (ML) have been proven suitable for interpreting vibrational spectra. While unsupervised ML allows exploring the data and to extract particle spectra from larger hyperspectral images [3], supervised ML was shown to be a reliable and quick tool for assigning particle spectra to a substance class [4]. Up- and downsides of database matching, supervised and unsupervised ML are critically discussed regarding their applicability for MP research. This includes time, staff and computational resource resources, as well as a discussion of evaluation methods for the techniques proposed, including tests on real-world data. The latter is a key factor in developing harmonized and comparable methods. 1. Weisser, J., et al., *The identification of microplastics based on vibrational spectroscopy data – A critical review of data analysis routines*. TrAC Trends in Analytical Chemistry, 2022. 148: p. 116535. 2. Munno, K., et al., *Increasing the Accessibility for Characterizing Microplastics: Introducing New Application-Based and Spectral Libraries of Plastic Particles (SLoPP and SLoPP-E)*. J. Anal. Chem., 92(3): p. 2443-2451. 3. Wander, L., et al., *Exploratory analysis of hyperspectral FTIR data obtained from environmental microplastics samples*. Anal. Meth., 2020. 12(6): p. 781-791. 4. Back, H.d.M., et al., *Training and evaluating machine learning algorithms for ocean microplastics classification through vibrational spectroscopy*. Chemosphere, 2022. 287: p. 131903

3.09.V-04 How to Test Weathered Microplastics of Various Sizes

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Although, the number of studies focusing on potentially harmful ecotoxicological effects of microplastics has been increasing, there remains a lack of environmentally relevant data. Mostly uniform and pristine materials are used for toxicity testing. At the same time, there is an ongoing discussion about potential size-related effects, assuming that the smaller the particles, the higher the potential effects on organisms might be. In our study, we addressed two issues: (1) more environmental realism by subjecting the test polymer particles to wet weathering (UV and shaking) and (2) potential size-related effects by applying a fractionation approach subsequently to the weathering. Appropriate characterisation of applied suspensions with regard to particle size, shape and number is the key for interpretation of toxicological data. In our study we investigated three types of polymers, low density polyethylene (LDPE) and polyethylene terephthalate (PET) and a consumer product. All materials were cryo-milled down to a range of below 250 µm. To mimic the weathering of polymer particles in the aquatic environment, polymer particle suspensions were exposed to UV radiation and stirring. For comparison, the corresponding original pristine particles were used. In order to test the potential size-dependency of toxicological effects, the samples were dispersed in water and fractionated by wet sieving. We received five fractions (> 140 µm, 140 – 60 µm, 60–40 µm, 40 – 20 µm, < 20 µm), whereas their mass amount was calculated. For quantification of particle sizes and shapes, dynamic imaging techniques were applied. A further advantage of these measuring method is that particles are counted. This gives the opportunity to consider both, number and mass-based concentrations in in-vitro studies. The effect of the different fractions of weathered and unweathered polymer suspensions on the 48 h immobilisation of *Daphnia magna* was assessed subsequently. It revealed little effect of polymer type, particle size, as well as aging status on toxicity in the concentrations tested. Dose metrics were challenging, as not for all fractions the same characterisation methods were applicable. Our study demonstrated that the fractionation approach works for all types of polymer particles, artificial microplastics and realistic materials such as consumer products. Further, it is applicable independently from the weathering status of the materials.

3.09.V-05 Microplastic Pollution Around Svalbard - CTD Samples Give First Evidence of Microplastic Distribution and Pathways Within the WATER Column

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Several studies have already revealed microplastic pollution in different matrices, e.g., snow, ice, water, and sediment in the Arctic environment around Svalbard. In contrast, knowledge on the microplastic distribution and transport pathways within the water column at different depths is still lacking. To address this issue, the Norwegian Polar Institute (NP) conducted a research cruise focusing on microplastic pollution in the Barents Sea in June 2021. Among other sample types and techniques to collect ice, water, and sediment samples, a CTD casts equipped with a rosette of Niskin bottles was utilized to collect water samples at different stations (N = 5) and various depths (0 – 1,430 m). Three replicates of 30L⁻¹ at each depth per station were taken to cover a sample volume of approximately 1m⁻³ to assess valuable results on microplastic contamination in this Arctic area. The collected water samples were directly sieved (50 µm) onboard to decrease the sample volume. The sieved and rinsed samples are stored at -20°C until further processing. For a reliable evaluation of the amount and composition of considered anthropogenically synthesized particles (including fragments and fibres), µRaman spectroscopy will be utilized for further identification. Procedural blanks and other safety measures were applied and considered to control and evaluate the potential contamination through the field and working environment (incl. ship and laboratories). This study focuses on acquiring a harmonized protocol to ensure no particle loss and the avoidance of an overestimation based on secondary contamination. Furthermore, this study will give first evidence on the microplastic distribution (quantity and quality) in different water depths of the Barents Sea and the west coast of Svalbard, including the Isfjord. In accordance, this analysis will be the first outcome out of this plastic dedicated research cruise, and thus the very first hint of the actual microplastic burden in different matrices around Svalbard.

3.09.V-06 Innovative Technology for In Situ Quantification of Nano and Microplastics in Water Media

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Plastic pollution is ubiquitous in the environment. Micro and nanoplastics pose a threat for the health of living organisms. Although the literature provides a wide range of analytical techniques for the identification and quantification of microplastics in environment samples until now, there is a not standardized protocols and the methodologies developed have drawbacks as time consuming, expensive and low robustness. Furthermore, for nanoplastics identification there are not practical methodologies for its application in routine analysis. Therefore, there is an urgent need for new robust automated tools for fast micro-nanoplastics identification in routine analysis for short-term decision-making. This work shows early-stage research of an innovative technology for in situ and automatic analysis of small microplastic (< 125 µm) and nanoplastics in water samples. A microfluidic chip has been computationally designed by means of COMSOL Multiphysics software considering complex processes involved in microplastics fluid dynamics. The device is working under the following principles: microfluidics to sort the micro/nanoplastics by size and concentrate the samples; staining of micro/nanoplastics with solvatochromic dye (Nile Red) and, finally, detection with fluorescence-based techniques. The fabricated device was made of poly (dimethylsiloxane) (PDMS) material since it is the most widely used silicon-based organic polymer due to its versatility and characteristics leading to very large number of applications. The fabrication of the microfluidic device was done with photolithography providing high precision and resolution. Validation of geometry of the device such as separators and focusers has been performed with PS microbeads of different size

standards. Additionally, we report on for the first time to the best of our knowledge the staining of microplastic particles using Nile Red as a dye for short time in aqueous media, never reported before. The device has been used successfully in a case study employing low-density polyethylene (LDPE) microplastics previously collected from La Pineda beach (located in Tarragona-Spain). The pellets were grounded and sieve by size, the smaller ones were selected and tested with a successfully characterization of fluorescent signals. Further research is being conducted to improve the promising performance of the fabricated device and to solve additional challenges.

3.09 Micro- and nanoplastics: Towards the harmonized analysis for monitoring, effect studies and risk assessment (Poster)

3.09.P-Mo145 The Power of Multi-Matrix Monitoring in the Pan-Arctic Region: Plastics in Water and Sediment

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Assessing Arctic ecosystems for litter and plastic pollution is high on the agenda of the Arctic Council and Arctic Monitoring and Assessment Plan (AMAP) nations. Coordinated efforts by all parties will contribute to a combined knowledge of the distribution and fate of this global pollutant and aid in identifying priority areas/regions where mitigation efforts should be focused. When we focus on our knowledge of microplastics, there are increasing reports of their presence in the Arctic. Yet, efforts to track microplastics have been uncoordinated with little follow-up on the state of the ecosystem where they were initially found. Further, the data that is currently available from these environmental compartments is sporadic, collected with different methods and processed with different approaches, generating results that are not currently comparable across the Arctic region. Without baseline data that are comparable across the Arctic region - collected from water bodies and sediment - it becomes far too complicated to compile the data required by policy and researchers who want to model the movement and consequences of plastic pollution. Through this work, we have used the framework of the AMAP to illustrate how scientists, governments, and Arctic Peoples can work together to address microplastic pollution. Specifically, our focus is on linking efforts together to build an understanding of this pollutant from the local scale to the full breadth of the Arctic. To achieve this, it will be necessary to obtain data from more than one sample matrix. Quantifying microplastics in sediment and water from the same region will facilitate a three-dimensional picture of microplastics, not only as a snapshot in time, but as an ongoing process of pollution within the Arctic. By implementing systematically designed and harmonised monitoring programmes and research activities to investigations of the rivers, beaches, seas, and seafloor along a track that links our communities to the deepest parts of the Arctic Ocean we can gain the most complete picture of the spread of plastic pollution to date. We highlight the latest technical recommendations for monitoring microplastic pollution across the pan-Arctic and consider how the challenges of conducting research in the polar environment may be overcome in this endeavour. Effort must be directed toward harmonizing these approaches and validating the extent to which data can be used in combination.

3.09.P-Mo146 Accurate Microplastics Identification From <500nm to mm's : Particle Shape/Size Artefact-Free Submicron IR and Simultaneous Raman

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Whilst the scientific and even public interest in microplastics continues to see rapid growth, thanks to the explosion of research in the field, there remains several key analytical challenges, limitations, even misconceptions and misunderstandings when it comes to determining which analytical techniques are most suited for microplastics analysis. The recent development of a new approach to IR microspectroscopy, termed "Optical Photothermal Infrared (O-PTIR)" spectroscopy has demonstrated a unique ability to generate submicron IR spectra in reflection mode (that are fully compatible with FTIR transmission mode) and thus to take the best properties of Infrared microscopy and Raman, whilst leaving behind all the issues of those techniques. Furthermore, this technique is also able to generate simultaneous Raman spectroscopy from the same spot, at the same time with the same submicron IR resolution. The application of both traditional IR and Raman microscopy to MPs suffers from a number of drawbacks, some of which are intuitively obvious (such as limited IR spatial resolution of >20µm), but other issues are not, hence many a MPs analysis practitioners may be unaware of the issues at play. In fact, the IR microscopy field has been clouded with the emergence of new instrumentation types, based on direct measurements with IR QCLs operating with both widefield (camera) based detection and also rapid scanning single point detection schemes. Whether an MP analysis is conducted with a traditional FTIR instrument or the emerging direct QCL based techniques, the issues, challenges, and pitfalls are all the same. Beyond the key issue of limited spatial resolution of direct IR microspectroscopy (>20µm), is the potentially very troublesome issue of particle shape and size dependent scattering artefacts which can interfere with the spectra so severely that accurate classification is made very difficult. In this presentation we will introduce, with examples, the various types of artefacts observed, from dispersive Mie-Scattering, diffuse/specular mixtures to band saturation issues. The issues with Raman spectroscopy, though it possesses excellent (and equivalent to O-PTIR) spatial resolution at the submicro level, are quite different, with it is often being plagued by auto-fluorescence interference and poor sensitivity, necessitating longer and sometime prohibitively measurement times. Through the use of a single frequency probe beam (532nm), the wavelength dependent dispersive scattering (Mie-scattering) issues

associated with variable IR probe wavelengths (such as in FTIR and direct QCL measurements) are circumvented. Additionally, the other issues often encountered with such methods of band saturation and competing specular and diffuse reflectance are also eliminated. The end result is a series of measurements that are now no longer confounded with particle morphology (shape/size) but are only dependent on their chemistry, which is what is needed for robust and repeatable MP analysis, independent of particle shape and size.

3.09.P-Mo147 Arctic Mammals and Plastic Pollution: Monitoring Efforts Directly Relate to the Monitoring Objectives

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Effects monitoring is a critical component for understanding the impacts of plastic pollution on the environment. Studies on mammals are important for addressing questions related to regional food security and safety. Given the important and prominence of mammals in the diets of many communities across the Arctic, effects monitoring should focus on species that are 1) consumed, and 2) experience high levels of plastic accumulation. The currently available knowledge in the Arctic has been mostly driven by opportunistic collections, and not specifically for monitoring purposes. Monitoring of litter and microplastics in the pan-Arctic requires a multi-matrix approach which can build on existing monitoring programmes and modify methods accordingly. The AMAP monitoring guidelines suggest that mammals might not be the priority target matrix, but they may be useful as part of a suite of tools needed to better understand pollution in the Arctic. Future monitoring programs should consider a range of monitoring objectives, and consider monitoring mammals as part of the suite of tools useful for monitoring plastic chemical additives and effects, as well as understanding the source and surveillance of litter and microplastics. That said, local or regional efforts are not being discouraged; rather further research is required to considering scaling to the pan-Arctic. Using mammals to study plastic pollution requires much larger sample sizes than typically collected for consumption, or for some other research programs. Here, the benefit is that hunter-collected samples can inform studies on plastics and plastic additives in relation to species regularly consumed by humans. Such assessments could also be conducted in parallel to the monitoring of plastic additives, preferably coordinated with other contaminant monitoring programmes and potentially associated biological effects. Local and Indigenous researchers and community representatives should be part of decision-making processes for priority species and research questions in their areas. The ethics of reporting back and publishing contamination results for wild food, even if the results are null or low, is an area of crucial importance and should play a central role in monitoring projects moving forward. Tissues regularly consumed by humans should also be selected to provide information on human exposure to plastic additives via the consumption of country foods.

3.09.P-Mo148 EUROqCHARM - Assuring Reproducible, Harmonised and Quality-Controlled Assessments of Plastic Pollution

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Plastic pollution (macro to nano) has become a global environmental and societal concern. Numerous protocols have been developed to monitor plastic debris, but these are rarely comparable. This has hindered the gathering of knowledge regarding pollution sources, development of monitoring programs, risk assessments, and the implementation of mitigation measures. To develop long-term solutions to reduce plastic pollution, it is essential to establish harmonised methodologies. EUROqCHARM, a European Commission H2020 Coordination and Support Action (CSA) project, is addressing this by critically reviewing state-of-the-art analytical methods and validating them through an interlaboratory comparison (ILC) study. Outputs will be integrated into recommended procedures for policy, legislation and risk assessment, as well as capacity building exercises, and knowledge transfer and dissemination. The EUROqCHARM consortium consists of 15 partners, a scientific advisory board composed of international experts, and a network of more than 25 associated laboratories. EUROqCHARM recognises that harmonisation for large-scale monitoring requires flexibility, comparability and reliability. As this is a CSA, our aim is to provide a cross-Europe and international platform to validate several methods for monitoring plastic in the environment and put forward recommendations for monitoring. In more detail, we aim to identify, test and optimise monitoring approaches through quality assured and rigorously validated methods based on current state-of-the-art techniques which cover all relevant environmental matrices. EUROqCHARM is focused on harmonizing and the possibility of standardising methods and reporting formats to facilitate data comparability and meta-level analysis on regional, national and international scales. EUROqCHARM is now entering an intensive phase with the results from the systematic review of more than 3000 papers being evaluated to identify commonly used and reproducible approaches. Moving harmonization forward, the identified methods will be incorporated into the

ILC taking place in 2022. By including multiple national and international organisations, and working groups currently participating in the proposal of harmonisation and standardised methods for research and monitoring, EUROqCHARM brings these players together to merge working group ideas and facilitate a framework for urgently required procedures for monitoring and assessment of plastics in our environment.

3.09.P-Mo149 Screening of Suspected Micro(Nano)Plastics in the Barcelona Metropolitan Area

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Plastics are recognised by the scientific community as an emerging risk for the environment and human health. The most important sources of plastic pollution come from industrial and anthropogenic activities (primary plastics). This plastic litter undergo continuous degradation processes (secondary plastics) generating a broad amount of microplastics (MPLs; particles size up to 5 mm) and nanoplastics (NPLs; plastic particles less than 1 µm). Main problems associated with micro(nano)plastics (MNPLs) are the lixiviate of contaminants used in their formulation, their small sizes, wide occurrence and long life in the natural environments. In addition, due to their properties, MNPLs can also enhance the transport of many organic chemical contaminants by adsorption/desorption processes, acting as a passive carriers of hydrophobic organic substances [1]. This work reports the micro and nanoplastics (MNPLs) with size ranges from 0.7 to 20 µm found in tap water samples of the Barcelona metropolitan area (BMA) collected during a sampling campaign carried out in September and October 2020. Tap water samples were collected from home pipes of volunteers distributed in the zip codes of the BMA. To identify and quantify the polymers in the samples, a suspected screening validated method based on high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS) using an advanced polymer chromatography column (Acquity APC XT45 1.7 µm) and atmospheric pressure photoionization (APPI) source under negative and positive conditions was used taking into account a previous method developed and validated in our research group for MNPLs in seawater compartments [1]. The acquisition was performed in full scan mode, and the subsequent tentative identification was based on the Kendrick Mass Defect analysis (KMD). The main results of the samples showed that polyethylene (PE), polypropylene (PP), polyisoprene (PI), polystyrene (PS), polybutadiene (PBD), polyamide (PA) and polydimethylsiloxanes (PDMS) were most commonly found. In particular, PE, PP and PA, while the polymers found at high concentrations were PI reaching 39 µg/L and PBD reaching 27 µg/L. A principal component analysis (PCA) was performed to assess if there are significant differences (confidence level 95%) between districts. Despite the fact that significant differences were not found among the studied area, there is a trend indicating that the samples collected at the west part of BMA presented a higher number of polymers and at higher concentrations.

3.09.P-Mo150 Monitoring Guidelines for Polymer Identification, Quality Assurance/Quality Control QA/QC and Data Reporting for Monitoring of Microplastics in the Arctic Environment

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The increasing pollution of the environment with plastics is of growing concern, also in the Arctic region. Larger plastic litter items, such as fishing gear or plastics bags, are a visible pollution issue in the environment. Their presence can have negative impact for economic factors like tourism or for recreational quality, while it further poses a threat to animals by entanglement and ingestions. In contrast, microplastics are not readily visible in most cases, but of concern for animal health and products for human consumption as accumulation in animal tissue can occur, including Arctic food items. For their analysis, these small plastic particles need a series of dedicated preparation steps, including sampling, sample extraction, clean up and advanced analysis in order to robustly identify and quantify them. Due to the small size of these microplastic particles and a variety of possible sample contamination sources, our study presents a set of general guidelines for their sampling, laboratory extraction and analysis. These guidelines outline and evaluate the different methods and tools available and include a series of recommendations. We start with a focus on general and specific quality assurance and quality control (QA/QC) measures related to microplastic sampling in different environmental matrices. Recommendations for QA/QC measures for the handling of samples in the laboratory, both on land or on ship, including an assessment on the scope and use of laboratory and procedural blanks are the second step. The final component of the study focuses on a general overview and discussion of suitable analytical tools for the identification, quantification and characterization of microplastics, considering a cost efficient and high throughput application. The recommendations proposed are anchored in the provision of mandatory data for reporting and in the generation of additional information that is of high value for scientists, stakeholders and legislators. Relevant data platforms are discussed with a view to their suitability to include Arctic data on microplastics.

3.09.P-Mo151 Current Status of the Quantification of Microplastics in Water

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We present here an overview of an inter-laboratory comparison (ILC) that aimed to investigate the current status of microplastic quantification in water and to support the development of microplastic reference materials. Measurement results were produced by almost 100 participating laboratories who also provided detailed technical information about the instrumental methods and experimental procedures used. The reported number and mass concentrations were evaluated in relation to the technical details provided and analysed for possible correlations. As a result, our study gives a broad picture of methods and procedures currently utilised in the measurement of microplastics in water. Sixteen different analysis techniques appeared in this ILC, but no (group of) methods could be identified that systematically seem to give results that are more consistent with the indicative ranges of expected values than others. The reported measurement data scattered around the centre values of the indicative ranges but the spread was quite large, even when looking at individual techniques. Furthermore, a great variety of experimental practices was applied, making it difficult to identify individual factors that significantly influenced the measurement outcome. Nevertheless, the outcome of this comprehensive study provides valuable input for further harmonisation and standardisation efforts towards reliable quantification of microplastics in water and other matrices.

3.09.P-Mo152 Meta-Analysis As a Tool for Combining Evidence of Micro and Nano Plastic Effects

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Micro- and nanoplastic particles (MNP) are found worldwide, even in the most remote regions, from deserts to the Arctic, and the number of studies reporting adverse effects of MNPs on organisms is increasing rapidly. The scientific literature supports policymakers and stakeholders in understanding and predicting these adverse effects and establishing an environmental risk assessment for MNPs. The increasing number of publications on MNP effects on organisms covers a wide variety of test species and MNP traits: for example, MNPs in these studies differ in polymer type, size, shape, and degradation stage; they are chemically modified, weathered artificially or tested in combination with surfactants or other plastic-associated chemicals; and in some studies the tested MNPs are produced directly from plastic products. To get a better overview of the general effects of MNPs, it is necessary to aggregate all this information in a combined analysis. One means by which such a data aggregation can be achieved is the use of meta-analyses. Meta-analyses combine statistical results or effect sizes of several studies to summarise the evidence in a particular topic and are widely used for example in medical and epidemiological research. Although meta-analyses have become more broadly used in ecotoxicology in recent years as well, their use in risk assessments is still limited. We discuss the importance of meta-analyses for aggregating evidence in ecotoxicological risk assessment in general and argue that datasets produced by systematic data extractions from the literature are a valuable resource for predictive modelling approaches. To demonstrate this, we use a dataset on effects of MNPs on *Daphnia magna* that we extracted from the literature and discuss how meta-analyses can help to identify MNP traits associated with toxic outcomes.

3.09.P-Mo153 Fate and Transport in Wastewater Treatment PLANTS: Adapting a Draft OECD Guideline to Assess Heteroaggregation of Micro- and NanoPlastics

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Recent research reveals various environmental scenarios (including road run-off and textile washing) in which microplastics end up in wastewater treatment plants (WWTP). Their ability to remove microplastic particles from influent via aggregation and adsorption processes to sludge is essential to reduce microplastic pollution of effluents and rivers. However, the scientific knowledge on heteroaggregation processes of microplastics is limited, which is why it is important to investigate to which extent microplastics are retained in the sewage sludge. Here we adapted a draft OECD guideline, that was originally proposed for WWTP removal of nanomaterials, to become a standardized process simulating the fate of micro- and nanoplastics in a WWTP. The process itself is focussed on heteroaggregation between natural colloids (sewage sludge) and microplastics only and does not include WWTP pre-treatments. The simplified and standardized process is applicable in the laboratory and is therefore suitable for a material comparison, providing a simple pre-screening possibility for potential retention efficiencies in WWTPs. The process includes standardization of sewage sludge from a municipal wastewater treatment plant for experimental reproducibility. PA-6 (< 5 µm) and two types of controls (anionic and cationic solid, insoluble polymer particles, made by emulsion polymerization) were monitored by a batch experiment based on mixing and sedimentation with and without sludge colloids, determining the remaining amount of polymer in the supernatant, which would be the effluent. Pyrolysis GC-MS was used for identification and quantification, in accord with the increasing use of pyrolysis GC-MS for environmental monitoring. We successfully developed a reliable and simplified process for simulating the fate of micro- and nanoplastics in wastewater treatment plants, including sample treatment protocols for pyrolysis GC-MS. The process in combination with suitable controls can serve for a material comparison and to determine heteroaggregation attachment efficiencies between micro- and nanoplastics and sewage sludge. However, the relevance of the measured retention still needs to be assessed against retention in real-world WWTPs. Additional settling processes and flocculating agents would increase retention, and could be added to the guideline in a tiered testing strategy.

3.09.P-Mo154 Determination of Microplastics in Soil and Water Samples From a Managed Aquifer Recharge System With Wastewater Treatment Plant Receiving Waters

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Plastic materials are extremely versatile due to their low density, low thermal and electric conductivity, and resistance to corrosion, which allow these materials to serve as a water and oxygen barrier. Their low price also contributes for their easy and widespread manufacture, where they are used in a wide range of applications from food packaging to medical and technological applications. Environmental conditions such as solar radiation, abrasion and diverse interactions with organisms, among many others, cause plastic to degrade and fragmentate into smaller particles commonly known as microplastics (MPs). MPs might accumulate in the natural environment, considering the low degradability of most plastics, thus their analysis and determination is of utmost importance. Wastewater treatment plants (WWTPs) have been identified as one of the highest sources of plastic release into the environment. In the present work, a simple analytical method for the simultaneous determination of MPs, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), and polyethylene terephthalate (PET), in water and soil samples from a managed aquifer recharge (MAR) system which receives water from a WWTP has been optimised and validated. The method is based on a density separation of the MPs using a salt saturated floatability solution, followed by an oxidative treatment using hydrogen peroxide in presence of iron (II) sulphate heptahydrate (Fenton's reagent) in order to eliminate the organic matter present in the samples. After the oxidation, the solution is allowed to settle, before collecting the supernatant, which is filtered through a 1 x 1 cm² silicon filter. Once particles are retained onto the filter, it is analysed by FTIR imaging, using a Nicolet™ iN™ 10 MX infrared imaging microscope. An exhaustive pretreatment of the sample is needed in order to obtain cleaner supernatants with less organic matter and sediments, thus improving the posterior characterisation by FTIR *imaging*. The improved method will be applied to the analysis of barrier material as well as water samples from the MAR pilot system.

3.09.P-Mo155 A Novel Methodology for the Removal of Microplastics From Deep Sea Sediments

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One of the main challenges in identifying and quantifying microplastics is extracting the microplastics from the matrices in which they are found. This is a feat that is currently lacking efficiency, reproducibility, and standardization throughout the field of microplastic research. Current methods for removing microplastics from the environment use inefficient glassware and expensive density separation devices that require transferring samples between glassware, with each transfer increasing the likelihood of sample loss. Harsh chemicals including Fenton's reagent, sodium hydroxide, and potassium hydroxide are often used to remove the organic matter in a sediment sample but can be damaging, potentially leading to the loss or misidentification of the microplastics. The objective of our study was to design a readily accessible and affordable density separation device (DSD) to optimize and harmonize research in the microplastics community. A DSD was built with two 1.5 inch diameter sight glasses connected by a stainless steel ball valve. In a novel approach to dealing with organic matter in the sample, a vacuum was applied prior to density separation which reduced the amount of organic plant matter in the sample by 93%. Stainless steel balls were used inside the DSD to mix only the bottom chamber in order to release plastic particles bound to sediment and increase recovery. Furthermore, all steps of the separation process, until filtering, take place inside the DSD, eliminating the need for sample transfer and greatly reducing the chances for sample loss. The recovery potential of our DSD was tested by spiking samples of deep sea sediment in triplicate with eight polymers (fragments of polypropylene (PP), high density polyethylene (HDPE), polystyrene (PS), nylon-6 (PA6), polyvinyl chloride (PVC), crumb rubber from tires (CR), and two fibers, polyester (PEST) and cellulose acetate (CA)) between 100 and 300 µm in size. Separation was achieved with 2.0 g/mL sodium polytungstate. Recoveries of all fragments exceeded 80%: PP: 81.6 ± 25.7%, HDPE: 96.1 ± 13.4%, PA6: 91.4 ± 19.2%, and PVC: 101.4 ± 9.7%. The recovery of CR was greater than expected (212.4 ± 115.3%) due to the fragmentation of the CR during processing. Fiber recoveries were lower than expected (PEST: 41.1 ± 35.6%, CA: 34.8 ± 8.3%) and require further investigation. Overall, the DSD has proven to be an efficient, easily accessible, and affordable solution to microplastic extraction.

3.09.P-Mo156 Determination of Microplastics in Macroalgae Using FTIR Microspectrometry

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Algae are an important source of food and a refuge for marine species because they form marine forests of huge ecosystem importance. They also emerged as an economic resource (food, cosmetics). However, it was reported that they could retain large amounts of microplastics (MPs). Further, they may transport MPs and, so, have implications for the food chain. This work focuses on developing a reliable method to characterize and quantify MPs in two types of matrices: seawater containing macroalgae and plain surface stranded algae. For this purpose, several digestion methods were tested, where from an enzymatic-oxidative protocol was selected and validated, with good results. It uses cellulase, protease and H₂O₂. Validation was made by preparing a mixture of 5 algae (usually sold in supermarkets; namely, *Undaria pinnatifida* spp, *Porphyra* spp, *Ulva* spp, *Laminaria ochroleuca* and *Himantalia elongate*). The two phases of seawater-containing algae were separated and subsequently digested. A comparison with a traditional method that washes the algae was made. It is concluded that the digestion procedure results in better recoveries than the washing protocol. In addition, studies were made to reduce the measurement time required when an IR microscope (Perkin Elmer Spotlight 200i) is used to characterize the suspicious particles. The characterization of the particles in a filter was

made in a two-stage approach. First, larger (>100 µm) particles were transferred to dedicated multi-well aluminium plates [1] (this is a quite fast step and the subsequent semiautomatic measurement with the microscope is straightforward) and, then, the filter (47 mm diameter) was scanned following a helical subsampling pattern. [1] López-Rosales A., et al., Development of a fast and efficient method to analyze microplastics in planktonic samples. *Marine Pollution Bulletin*, 168:112379. 2021.

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3.09.P-Mo157 Recent Developments, Applications, and Future Perspectives for siMPle, a Freeware Tool for the Manufacturer-Independent Analysis of Microplastics

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Microplastics analysis is an emerging and challenging topic for laboratories worldwide due to the various steps involved. These steps range from sampling, work up/purification, to analysis, and each has its own challenges. Additionally, the final analytical step can be carried out using different methods based on spectroscopy or thermoanalysis available, which provide different data quality, comparability, and mass or number-related results. Among these, the analysis via Raman or Fourier-Transform Infrared (FTIR) spectroscopy is often applied to determine the chemical nature of these particles. In the case of FTIR imaging, the procedure allows the measurement of complete filter areas independent of human bias in a short amount of time. In the scope of recent approaches to determine standardized operational protocols (SOPs), access to comparable data determination is in many cases difficult due to a large number of different manufacturers as well as commercial software solutions and spectral libraries available. Hence, siMPle was developed to overcome this limitation and allow the harmonization of data analysis. It allows the analysis of datasets measured on different machines from the manufacturers Agilent, Bruker, DRS Day Light Solutions, Perkin Elmer, and Thermo Fisher (further imports in development). In the current stage, every spectrum can be selected individually or analyzed via two pipelines for the automated analysis, which can handle a large dataset with more than 11 million spectra with relative ease. This study will present recent developments of the software tool focusing on usability, improved function ability, and spectral libraries. The generated data by this additional tool was benchmarked and validated in accordance with standard analysis approaches and libraries within siMPle to allow a harmonized comparison of results. In addition, the influences on calculation time for a reference data set containing 1 million spectra were monitored and evaluated. This tool and its further updates and spectral libraries are available as Freeware on www.simple-plastics.eu for the harmonization of MP data analysis for spectroscopic data.

3.09.P-Mo158 Fast Microplastic Classification Using µFTIR and Random Forest

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Fourier-transform infrared micro-spectroscopy (µFTIR) is a commonly used non-destructive probing technique for Microplastic (MP) analysis. It allows for both chemical identification and geometric analysis of MP particles in a MP sample. Analysing the spectral information of a MP sample renders data acquisition and analysis challenging and time consuming. One promising path for fast and reliable automated analysis of µFTIR data from MP samples are Machine Learning (ML) classification methods such as Random Forest (RF). RF is a supervised ML method, meaning that it requires training with labelled data, i.e. spectra that have been tagged as a certain type of MP by a human expert or equivalently reliable means. In the present work, µFTIR data from lab-created MP samples are semi-automatically labelled. This data consists on labelled MP spectra and is used to generate a training dataset and an independent test dataset. The RF forest is trained until convergence using only the training dataset, the training progress is validated using the test dataset. Another test case is the measurement of sediments from environmental wastewater samples using fast MP detection. The RF classifier is able to detect the MP types that it has been trained to classify, including some MP particles that have been missed by using other state-of-the-art classification methods.

3.09.P-Mo159 Investigation of Microplastics in the Tropical Indian Ocean Using Laser Direct Infrared (LDIR) Chemical Imaging and Microwave-Assisted Sample Preparation

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In this study we introduce a novel in-house-built fractionated water filtration system (Geesthacht Inert Microplastic Fractionator, GIMPF) to sample large water volumes and extract high loads of suspended particulate matter out of water bodies. It was thoroughly tested for its efficiency to retain small-sized microplastics ($d > 300 \text{ ?m}$ and $10 \text{ ?m} < d < 300 \text{ ?m}$) with metal doped model microplastic particles using Inductively Coupled Plasma – Tandem Mass Spectrometry (ICP-MS/MS). The GIMPF was applied on real environmental samples taken during SO270 cruise (*RV Sonne*) in the tropical Indian Ocean. 21 Samples ($10 \text{ ?m} < d < 300 \text{ ?m}$) were subjected to a microwave-assisted one-pot sample preparation protocol. The validated microwave-assisted matrix removal protocol showed quantitatively high matrix removal rates ($94.3\% \pm 0.3\%$ (1 *SD*, $n = 3$)) using a certified reference material and high recovery rates ($95\% \pm 4\%$ (1 *SD*, $n = 3$)) for model microplastics, using in-house HDPE and PET model particles in the size range between 20 µm and 63 µm. Furthermore, model microplastic particles were investigated using Scanning Electron Microscopy (SEM) analysis after and before matrix digestion, which showed no significant changes in surface morphology and particle integrity. Microplastics were chemically identified and physically characterized using recently introduced Laser Direct Infrared (LDIR) Chemical Imaging based on a quantum cascade laser (QCL) source. This novel infrared imaging technique enabled fast and automated analysis of each entire sample, combined with a new efficient sample preparation

method, and reduced uncertainty introduced by any extrapolation. Microplastic particle number concentration of 21 analyzed tropical Indian Ocean samples (20 μm – 300 μm) ranged from 8 to 132 particles/fibers m^{-3} (mean: 50 ± 30 MPs m^{-3}) along the sampled transect at 6 m depth. The most abundant polymer clusters were acrylates/PU/varnish (49%), PET (26%), PP (8%), PE (4%) and EVA (4%). 96% of the microplastic particles/fibers had a diameter < 100 μm . The samples were collected along a previously unexplored transect and cover a large ocean area for which, according to our current state of knowledge, there is a poor data situation. Though inter-study comparison is difficult, the sampled area exhibits a high contamination with marine plastic debris compared to other open ocean regions. A distinct spatial trend was observed with an increasing share of the particle size class 20 μm – 50 μm from east to west.

3.09.P-Mo160 Microplastics in Beach Sands and Sandy Sediments: Comparative Analysis of Infrared and Fluorescence Methods

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Microplastics (MPs) are plastic particles of less than 5 mm that accumulate throughout ecosystems. The scientific community aims to standardize methods for the identification and quantification of MPs. This study proposes a method for the analysis of MPs in beaches and sandy sediments, based on visual sorting for MPs larger than 0.5 mm and a density separation method with a ZnCl_2 solution followed by Fenton's reagent oxidation for MPs smaller than 0.5mm. Infrared spectroscopic techniques, such as ATR-IR and micro-FTIR, and fluorescence microscopy with Nile red dye are used for polymer type identification. This method was applied to 32 samples, including 26 beaches and 6 sediments distributed along the Catalan coast (western Mediterranean). MPs larger than 0.5 mm were mostly fragments and fibres of polypropylene (50%) and polyethylene (31%), while MPs smaller than 0.5 mm were mostly fibers (400 μm in length) and particles (100 μm in diameter) of cellulose derivatives (42%), polyethylene (18-26%), polyamide, polyvinyl chloride, etc. Along the Catalan coast, the highest incidence of MPs is in the province of Tarragona, at points where rivers and wastewater treatment plants discharges coincide. In addition, urban and industrial pressure, marine currents, coastal line morphology and fine granulometries are conditioning factors of the distribution of MPs, making it heterogeneous along the coast. High pollution levels were detected in Ebro's Delta region such as the Bay of Fangar with 8406 items/kg dw as well as in the city of Tarragona with hot spots with more than 10000 items/kg dw. The proposed method is a strong candidate to be a standard method in microplastic analysis. However, the study found up to 30% items/kg dw difference between fluorescence microscopy and quantification by micro-FTIR spectroscopy on fine sand beaches, which should be further studied to ensure data quality.

3.09.P-Mo161 Evaluation of Weathering of Freshwater Environmental Microplastics by Fourier Transform Infrared Spectroscopy

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Fourier transform infrared (FTIR) is a spectroscopy technique widely used to identify organic materials. It has recently gained popularity in microplastic (MP) pollution research to determine the chemical composition of unknown plastic fragments, but it could also be used to evaluate the degree of ageing of MPs collected from the environment. In this context, the principal aim of our research has been to qualitatively evaluate the natural weathering of environmental MPs collected in an Italian freshwater body (Ofanto river) during Spring 2018 using ATR-FTIR technology. FTIR spectra were acquired using a Nicolet Summit FTIR (ThermoFisher Scientific) equipped with an Everest ATR with a diamond Crystal plate and a DTGS KBr detector (wavenumber range 4000-500 cm^{-1} , 32 scans per spectrum, spectral resolution of 4 cm^{-1}). The degree of ageing was assessed using three different indices known to be related to changes in MPs: Carbonyl Index (CI), Hydroxyl Index (HI) and Carbon-Oxygen Index (COI). The three indexes were calculated as the ratio of the absorbance peaks at 1770-1700/1495-1423, 3553-3021/1504-1467 and 924-1197/2987-2866 respectively. The overall results showed that the regions reflecting ageing-related changes (hydroxyl groups, peaks from 3100 to 3700 cm^{-1} , alkenes or carbon double bonds, 1600 and 1680 cm^{-1} and carbonyl groups, 1690 and 1810 cm^{-1}) appeared greatly modified compared to the pristine materials in each type of MPs. Moreover, new peaks are present almost all in the fingerprint region (1500-500 cm^{-1}). Differences in CI, HI and COI were evidenced among the different morphological MP shapes. On the one hand, the CI calculated for the PE pellet samples showed values ranging from 0.05 to 0.26 with a mean value of 0.17 ± 0.10 . The majority of samples (57%) presented a CI with values between 0.16 and 0.30. On the other hand, fragments presented slicer modifies in the carbonyl region with CI values lower than pellets (0.05 ± 0.05). This index is helpful to evaluate the degradation of PE MPs by UV light, and it increases with enhancing residence time in the environment. Conversely, fragments showed greater values of HI (5.90 ± 2.57) and COI (1.04 ± 0.48) than pellets, as well as lines, which presented the maximum value of HI (11.51). HI is attributed to the bond vibrations of hydroxyl, carboxyl or phenol groups, while COI is frequently attributed to the vibrations of C-O bonds found in carbohydrates, as well as to alkanes, secondary alcohols and ketones.

3.09.P-Mo162 Development of a Reliable Method to Analyze Microplastics in Fish Stomach Using an Infrared Tunable Quantum Cascade Laser System

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The presence of microplastics (MP) in fish stomachs causes great environmental concern due to the damage they cause to the specific individual and the possibility of trophic transfer. Infrared spectrometry is a common workhorse for chemical characterisation of MPs, but it is time-consuming. In this work, a IR device based on a quantum cascade laser is used to accelerate the characterisation step (Agilent 8700 Laser Direct Infrared (LDIR) chemical imaging system). Its novelty poses new challenges

for sample processing and particle handling, as the particles must be unfolded on a reflective slide without losing representativeness. This study compares three sample digestion protocols (alkaline-oxidative with H₂O₂, alkaline-oxidative with NaClO and enzymatic-oxidative) and three different procedures for transferring the contents of the filters to the reflective slides (manual, total evaporation, partial aliquots). A simplified enzymatic-oxidative digestion combined with a Syncore® automatic evaporation system is finally proposed as a reliable method. Fortified *Scomber scombrus* samples were studied, with recoveries ca. 100 % for MP >500 µm, ca. 90 % for MP > 200–300 µm and around 75 % for 10 µm thin microfibrils. **Acknowledgements:** Thanks are given to the European Union Funding for Research&Innovation (H2020-LABPLAS project, Grant Agreement No. 101003954 H2020-SC5-2020-2), JPI-Oceans and the Spanish Ministry of Science and Innovation (MicroplastiX project, PCI2020-112145). The Galician government is also acknowledged (Xunta de Galicia, Grant ED431C 2021/56).

3.09.P-Mo163 Effect of Weathering in the Leaching of Alternative and Conventional Additives From Microplastics to Seawater

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In the last years there was an increasing concern about the presence of plastic additives in the marine environment. Many of the functional plastic additives are classified as toxic substances and included in REACH¹. In this work, the migration of conventional (BHT, Chimassorb2020, TCPP, triclosan and diisodecyl phthalate) and less toxic alternative additives (?-tocopherol, quercetin dehydrate, lawsone, and Grinstead® Soft-N-Safe) from microplastics to seawater was studied using an accelerated migration method by pressurized liquid extraction. 3 petroleum derived plastics (PA, PVC, LDPE) and a bioplastic (PHB) were considered, both pristine and weathered, to study the effect of the weathering on the lixiviation of additives. Pristine polymers, LDPE and PHB showed in general higher migration than PA and PVC. The additives with a highest migration rate (about 10%) were TCPP and lawsone. In general, the plastic weathering decreased the additive migration and in some cases caused their degradation. Some degradation products of lawsone, triclosan and BHT were detected, which demonstrate that also the additives can be affected by the plastic weathering in the marine environment. This is quite relevant and further studies should be carried out, since some of these degradation products can be more toxic than the original additive. Considering the migration capacity and the toxicity of the additives, the conventional additives triclosan and BHT are the main contributors to the potential toxicity of plastics among the studied and the alternative additives tested seem to be a good choice to replace them. Finally, the substitution of conventional polymers by the biodegradable biopolymers could not be always a good alternative, since additives leachates easily from PHB (higher additive migration than PA and PVC), unless innocuous additives are used in the formulation. ¹EU, 2011. Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. Support: The Spanish Inter-Ministerial Science and Technology Commission and ERDF through ARPA-ACUA (CTM2016-77945-C3-3-R) project; the Spanish Ministry of Science and Innovation through JPI_Oceans 2019 MicroplastiX (PCI2020-112145) and CHEMPLAS (PID2019-108857RB-C3/AEI/10.13039/501100011033) projects. Program of Consolidation and Structuring of Units of Competitive Investigation SUG (Xunta de Galicia) cofinanced by ERDF-Operative Program of Galicia 2021-2024 (ED431C 2021/56).

3.09.P-Mo164 Different Software for Different Samples? Comparison of Two Automated Approaches for the Fast Analysis of Microplastics With Raman Microspectroscopy

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Raman microspectroscopy in combination with optical particle detection is a commonly used approach to identify and quantify microplastic (MP) particles in a wide range of samples. This particle-based approach often comprises of the steps optical image acquisition, particle detection, Raman scan, spectra analysis and result evaluation. To speed up analyses, software packages have been developed that provide all these steps in one application. While these packages include the same basic functionality, they differ in speed, accuracy and other functionalities that favor, for example, their application in certain MP size ranges over others. Here we present example analyses of MP particles in environmental and bottled water samples. We compare the results in the size ranges > 50 µm, >10 µm and > 4 µm obtained in parallel with the commercially available software WITec ParticleScout and the python-based GNU GPL-licensed GEPARD, which was developed at the IPF Dresden. ParticleScout as the proprietary software of the Raman microscope manufacturer WITec fully utilizes their instrument's capabilities, which results in optimized analysis speed, precision and ease of use. As an open source software GEPARD can be adapted to a broader range of instruments. In contrast to ParticleScout, it provides a unique annotation module for correcting inaccuracies in the particle detection with regards to, e.g., particle shapes, sizes, or over-fragmentation. For the two software packages, we discuss performance trade-offs between speed and accuracy for samples with varying particle size ranges and shapes. We also present how we combine GEPARD and Particle Scout to optimize the workflow and result accuracy.

3.09.P-Mo165 Advanced Image Recognition As Requirement for a Valid Analysis of Very Small (1--10 Mm) Microplastic Particles

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Microplastic (MP) particles (polymer fragments, 1µm—1mm) are found in a multitude of environmental samples. Reliable high throughput methods for quantification of the lowest size fraction within representative particle numbers, however, are still needed. In principle, Raman microspectroscopy (RM) is suitable for identifying particles down to 1 µm when deposited on a filter. *TUM-ParticleTyper*, an open source software, has been developed for detection of particles down to 10 µm *via* recognition in darkfield

images [10.1371/journal.pone.0234766], where measurement points (one per particle) are initialized for subsequent automated quantitative RM analysis. Here, we report on image processing methods that aim at improving the recognition performance further, thus enabling reproducible results down to 1 μm (maximum Feret diameter) under varying conditions. When advancing the methodology towards lower particle sizes, suitable methods of image processing are required to enable a correct detection despite influences of the instrumental framework. Specifically, construction-related contrast differences in sample illumination are problematic when using different objectives even if each of them may provide suitable magnification (50x or 100x). Here, filtering in the Fourier domain provides a solution, as it not only reduces the contrast of the filter pores that otherwise might interfere, but also preserves the sharpness of particles. Also, refined methods are needed to reduce false positive (e.g. morphological opening, median blur) or false negative results (e.g. top-hat transform). To separate agglomerates, watershed transformation is normally used, which is, however not reasonable when it needs to be applied to the whole sample involving too many small particles. Instead, an algorithm is applied here that considers only those objects whose convexity surpasses a threshold, which is derived beforehand by applying Otsu's algorithm to the convexity distributions of single particles and agglomerations after manual classification. Preliminary results show an increase in particle numbers to a value similar to the total number of particles obtained from manual subdivision. In combination, these advances provide the foundation for an improved image recognition in terms of useful and representative lateral positions for successive automated RM measurements. Ongoing developments aim to extend the applicability of the *TUM-ParticleTyper* approach for particles smaller than 10 μm .

3.09.P-Mo166 Micro Raman Analysis of Micro- and NanoPlastics in Drinking Water: From Number to Mass

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Lack of standardization still affects the microplastics science as a whole, and this is especially true for Raman microscopy applications in the field. In particular, calculating the particles' masses from Raman data remains challenging, though necessary in order to provide more significant evidences. The current work aims to exploit Raman microscopy in order to analyze micro- and nanoplastics in drinking water samples from a local facility, and to propose an assumption capable of allowing the calculation of particles' masses. In addition, a novel analytical protocol for micro- and nanoplastics analysis with Raman microscopy is presented. The main points of the research are the following: A sample preparation protocol for drinking water is presented. Large volumes (ca. 1 cubic meter) can be sampled through low-mesh size filters, and the retained particles can be concentrated in small volumes; Samples are deposited through a custom-made device on a proper Raman substrate; Relevant size parameters (major and minor axes) are obtained from visible image analysis of the deposited sample, and from them the volume of each particle is calculated assuming them as ellipsoids; According to the Raman spectrum of each particle, the pristine polymer's density is associated. The authors stress the novelty of the work, which is, at the best of their knowledge, the first of its kind.

3.09.P-Mo167 Chemical Mapping of Environmental Tyre Wear Microplastics by Py-Gc-Ms

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Tyre wear particles (TWPs) not only are assumed to be the most dominant source of environmental microplastics but also act as a source of various organic micropollutants, many of which are likely still unknown [1,2]. Most commonly, polymer identification in environmental samples is performed by non-destructive spectroscopic techniques, like Fourier transform infrared (FTIR) or Raman spectroscopy, but thermoanalytical methods such as pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC-MS) is the most promising technique for microplastics quantification [3]. A successful thermal desorption (TD-GC-MS) and pyrolysis (Py-GC-MS) method for the simultaneous characterization of organic additives/pollutants and main tyre composition was applied to used tyres. Knowledge of the chemical composition of TWPs, and the transformations that these compounds undergo with weathering, will help to better identify and quantify these particles in environmental studies. A weathering study of tyres in the marine environment has been carried out to monitor changes in additive composition and chemical structure. Besides styrene butadiene rubber (SBR), main component of TRWs which is used to Py-GC-MS quantification, hundreds of compounds as benzene, alkyl-benzenes, toluene or polycyclic aromatic hydrocarbons (PAHs) were identified. [1]Goßmann I. et al, Car and truck tire wear particles in complex environmental samples – A quantitative comparison with traditional microplastic polymer mass loads, *Sci. Total Environ.* 773 (2021) 145667. [2]Müller K. et al, Probing the chemical complexity of tires: Identification of potential tire-borne water contaminants with high-resolution mass spectrometry, *Sci. Total Environ.* 802 (2022) 149799. [3]Lauschke I. et al., Evaluation of poly(styrene-d5) and poly(4-fluorostyrene) as internal standards for microplastics quantification by thermoanalytical methods, *J. Anal. Appl. Pyrolysis.* 159 (2021) 105310. This work was supported by the European Union Funding for Research&Innovation H2020-LABPLAS project (Grant Agreement No. 101003954 H2020-SC5-2020-2), JPI-Oceans Program 2019 and the Spanish Ministry of Science and Innovation MicroplastiX project (PCI2020-112145) and by the Spanish Ministry of Science and Innovation 'ChemPlas' project (PID2019-108857RB-C31). The Program 'Consolidación e Estructuración de Unidades de Investigación Competitivas' of the Galician Government (Xunta de Galicia) is also acknowledged (ED431C 2021/56).

3.09.P-Mo168 Microplastics Analysis Throughout a Large Water Supply Network in Barcelona Urban Area by Py-Gc-Ms

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The consumption of plastics in Europe was 57.9 million tonnes in 2019 and has increased progressively over the years. Today, the

contamination derived from plastic represents a global threat and a matter of political, social and health concern. Many studies have shown that microplastics are widely distributed in the environment. Since most surface waters may be used as drinking water, the possible presence of microplastics in drinking water despite the treatments applied in DWTPs, is a matter of concern. However, the lack of standardized analytical methods leads to a discrepancy of the results between different studies. In the field of water policy, microplastics will be included in the watch list of the future European drinking water directive 2020/2184, once a harmonised methodology has been adopted. Thermoanalytical methods as pyrolysis-gas chromatography coupled to mass spectrometry (Py-GC-MS) provides the identification and the mass quantification (in terms of μg) about the concentration of the microplastics. The objective of the present study is to develop a fast, quantitative and suitable for routine analytical method based in Py-GC-MS, to determine the microplastics concentration in drinking water samples. The developed analytical methodology was applied to determine the concentrations and the distribution of microplastics throughout the drinking water supply network of Barcelona urban area, that provide drinking water to 3.000.000 inhabitants.”

3.09.P-Mo169 Quantitation of Tire Rubber Particles by Pressurized Liquid Extraction Coupled With Pyrolysis Gas Chromatography-Mass Spectrometry

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Tire and road wear particles (TRWP) pollution is an important problem because might generate health issues in humans and cause ecological damage. Furthermore, it is one of the largest sources for the distribution of microplastic particles in the environment. The complexity of this type of pollution is clear from the large variation in materials content: typically, 6 different rubber polymers are used for tire production. Moreover, the total mass of a tire generally consists of only 40-60% of natural and synthetic rubber. The remaining mass is composed of black carbon, vulcanization agents(1-2%), salts, heavy metals (5%), fillers (20-35%), process oils (12-15%), and other additives. Due to the complex mixture of these components, the characterization and quantification, of the rubber polymers, especially in environmental matrices, is challenging. There are many techniques to analyze rubber particles and pyrolysis gas chromatography-mass spectrometry (py-GC-MS) is a powerful tool for this purpose and probably one of the best for the quantitation in human and environmental matrices. The pyrolysis of tire particles generates different products (pyrolysates) that can be used as markers in the identification and quantitation of rubbers. In particular, the two main compounds that are generally utilized are vinyl cyclohexane (for styrene-butadiene rubber and butadiene rubber), and dipentene (also known as D-limonene for natural and isoprene rubber). However, recent studies show that the uncertainties of the results using only these two products can be remarkably high, especially when considering the high variability in the composition of different tires. We have developed a selective analytical method for the extraction and analysis of tire particles in environmental matrices. Quality control (QA/QC) parameters which include extraction efficiency (recovery), method specificity (pyrolysis temperature optimization and unique pyrolysate selection), and sensitivity (LOD/LOQ). The method has been characterized using different calibration curves produced by extracting tire samples using accelerated solvent extraction (ASE) and using an internal standard to balance the responses of the different pyrolysis products. This method will be used as a base for further investigations, in particular, for the characterization and quantitation of TRWP polymers in human matrices like blood and environmental samples such as air and soil.

3.09.P-Mo170 Quantification of Microplastics by Pyrolysis Coupled With Gas Chromatography and Mass Spectrometry in Sediments: Challenges and Implications

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Over the last few years, there has been a growing interest in studying microplastic (MP) contamination in freshwater systems. However, the knowledge on MP contamination is still insufficient and the developed analytical methods do not allow to establish a precise mass balance between the different environmental compartments. Generally, MP are analyzed by Fourier transform infrared microscopy (FT-IR) or Raman. These spectroscopic techniques provide information on the morphology of MP and an estimate of their number above a minimum size (25 μm), but do not provide a mass quantification of MP. Pyrolysis coupled to gas chromatography and mass spectrometry (Pyr-CG-MS), which was mainly used until now to identify the nature of MP in environmental samples, has recently been proposed to quantify them. This method allows both the simultaneous identification of polymers, without size limit, and their mass quantification. To date, this quantification of MP by Pyr-CG-MS is generally done by external calibration, without considering potential matrix effects. The matrix effect related to the analysis of MP in river sediments, for which it is difficult to totally isolate MP from the residual mineral and organic fractions, has been studied in floodplain sediments. This presentation aims to demonstrate and quantify this matrix effect through various experiments, and its implication on the study of MP in sediments.

3.09.P-Mo171 A Thermodynamic Method to Improve Microplastic Analysis of Common Plastics Using DSC

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The occurrence of microplastics in environmental compartments is a topic of upcoming risk assesment. The understanding of fate and pathways of microplastics in the environment is based on the environment compartment, enrichment processes and the analytical methods. An environmental monitoring is needed, which is provided by a reliable, cost-efficient and fast analytical

process. Therefore, the microplastic analysis by Differential Scanning Calorimetry (DSC) in combination with an electrostatic separation and density separation is a promising method to fulfil these requirements. The quantification and identification of microplastics by DSC depends on their thermodynamic properties. The polymer specific signals of melting, crystallisation and glass transition can be obtained by define heating and cooling regime in DSC measurements. The six common plastics named PE, PP, PET, PS, PVC and PA are subject of our study. The analytical properties of the method, e.g. the limit of quantification is discussed elsewhere. So here we want to show some limiting conditions for overlapping signals and furthermore present thermodynamic methods to overcome limitations of the applied method. Here, three different overlaps of signals are investigated. First, an overlap of two glass transition temperatures (PET, PVC). Second, an overlap of a glass transition and a melting peak (LD-PE, PS) and third, an overlap of different melting and crystallisation peaks (PET, PA). The glass transition is a kinetic process. This allows the signals of glass transitions in the thermogram to be specifically influenced by different heating and cooling rates. Thus, relaxation peaks can be obtained, which allow the identification or quantification despite overlapping signals. The signals from overlapping melt and crystallization regions of semi-crystalline polymers behave additively. Consequently, a clear assignment to a specific polymer can be generated from the information of the thermograms. These properties of semi-crystalline polymers thus correspond to a thermodynamic fingerprint. The results shown in this study improve the cost efficient analytical method for the determination of microplastics by DSC. The reliability of the method is increased. The adjustment of heating and cooling rates influences the time required for an analysis.

3.09.P-Mo172 Application of Electrostatic Separation and Differential Scanning Calorimetry for Microplastic Analysis in Particulate Matrices

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Microplastics in environmental compartments are a topic of growing concern. Time-consuming processes and analytical methods hamper the understanding of occurrence and fate of microplastics in the environment. Therefore, a fast, reliable and cost-efficient method for environmental monitoring is required. Most of the commonly used methods do not meet all requirements. They are highly time-consuming or may not give immediately confirmation of the polymer. Here we want to present an approach for microplastic analysis in particulate matrices combining electrostatic separation (corona drum separator, Hamos, Germany), density separation (potassium iodide solution, $\rho = 1.55 \text{ g/cm}^3$) and differential scanning calorimetry (DSC, Netzsch, Germany). Sand and river sediment samples were spiked with microplastic particles of ultra-high molecular weight PE (PE-UHMW), PET and PCL in the size range of 100 to 200 μm . Microplastic concentrations from 1 to 150 mg/kg were investigated. Subsequently, DSC data with phase transition temperatures and enthalpies allow identification and quantification of the polymer type. The recovery of microplastic was compared for different particulate matrices and polymer types. The electrostatic separation removed above 99 percent of the initial matrix from sand samples. In river sediments, above 90 percent of the initial matrix was removed, depending on the particle size distribution, bulk density and total organic carbon. Density separation increased the sediment removal to above 99 percent for all investigated matrices. The recovery of PE-UHMW was determined as 91 ± 5 percent in sand for concentrations between 40 to 100 mg/kg. The recovery was lower for river sediments than for sand. The recovery of PET and PCL were both above 80 percent. The recovery after density separation showed higher precision than the values after electrostatic separation. Additionally the presented combination of separation methods reduces to volume of necessary density solution. The first electrostatic separation step removes the majority of the matrix, hence the amount of density solution was reduced by 80 percent. Moreover, one sample can be analysed in 8 hours (waiting periods not included). This shows the economic advantages of this method for a comprehensive environmental monitoring of microplastic in river sediments. Our approach has the potential as a reliable, fast and cost-efficient alternative to methods commonly used like pyrolysis GCMS.

3.09.P-Mo173 Improvement of Retention in a Solid Phase for Thermoextraction-Desorption/Gas Chromatography/Mass Spectrometry (TED-GC-MS) in the Analysis of Microplastics

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To trap thermodecomposition products during pyrolysis in Thermoextraction-Desorption/Gas chromatography-mass spectrometry (TED-GC-MS), Gerstel twister stir bar coating with PDMS is usually used, being mainly suitable for non-polar compounds. Thermodecomposition products from microplastics are mostly non-polar, but some products may have polarity and are not properly retained in a PDMS sorbent, so to improve adsorption of thermodecomposition products of microplastics with a certain polarity a carbon adsorbent is used in this study being suitable for both polar and non-polar compounds. Furthermore, carbon materials usually have slit-type pores that are optimal for the adsorption of aromatic compounds, the nature of which is most of the decomposition products of microplastic.

3.09.P-Mo174 New Insights From Td-Gc-Ms Data on Microplastics in Salt Samples

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Detecting and analysing microplastics in the environment is challenging. The method must be able to detect and distinguish between a large variety of polymers. Additives, such as hardeners, flame retardants and preservatives, used during the manufacturing process and other compounds, that can adhere to the surfaces of microplastics from their environment, can affect their toxicity assessment. These additives could be used to link the microplastics found in the environment to their original use or potentially even back to the manufacturer. In this study, various salts were sampled and filtered and analyzed for their

microplastic content. TD–GC–MS was used to identify and measure which concentration of the plastic PS, PET, PVC and Nylon 6 had been incorporated into the crystalline structure of the salt during evaporation. By using direct desorption and backflushing technology, a large sample size could be easily isolated from the lab environment during analysis while ensuring a large range of volatile organic compounds (VOCs) including the marker compounds for four polymers could be trapped and analyzed. The VOC-profile of the microplastics not only gave information about the polymer and its concentration within the sample, but further information about the toxicity could be extracted. This enabled the determination and tentative identification of additional compounds in the samples, such as dimethyl ether, acrolein and cyclopentane. These are used in the process of manufacturing plastics, so could assist with identifying the source of the plastics. They also tentatively identified bisphenol A. Research suggests that BPA may be an endocrine disruptor in humans, making it a compound of interest when assessing toxicity. For the four analysed polymers distinct marker compounds could be identified and calibration curves determined for each polymer. In all of the real-world salt samples microplastics were identified, highlighting the ubiquitous nature of the microplastic contamination. Especially low-density polymers, which can be found in the surface of the ocean, can easily make it into natural-origin sea-salt, but potentially into surface feeding sea life as well. This further information about the origin of the polymer, as well as the origin of the microplastic particles can be gathered by investigating the full VOC-profile obtained by TD-GC-MS. This allows both polymer and particle source characterization simultaneously.

3.09.P-Mo175 Quantification of Polyvinyl Chloride Microplastics Via Solvent Extraction and Combustion Ion Chromatography

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Thermal methods have largely become established in the environmental analysis of polymers. They offer good opportunities for both identification and quantification of a wide variety of polymer types in aqueous and solid environmental samples. For example, methods for analyzing different plastics such as polyethylene, polypropylene or polystyrene in different environmental matrices by means of pyrolysis gas chromatography / mass spectrometry have been developed. However, one challenge with quantification *via* pyrolysis products is to identify specific marker substances for each plastic that are not produced by naturally occurring substances. The thermal decomposition usually results in relatively unspecific substances, which makes it difficult to quantify several polymers in one sample at the same time. State-of-the-art pyrolysis methods for polyvinyl chloride (PVC) analysis are using benzene as quantifier. However, benzene can also be formed during the pyrolysis of polyethylene terephthalate or organic substances such as cellulose. A promising alternative is the indirect quantification *via* hydrogen chloride (HCl), which is released during thermal decomposition of PVC. In this study the applicability of combustion ion chromatography (C²IC) for determination of PVC in environmental was evaluated and showed a good linearity and sensitivity. A fundamental problem, however, is that HCl can also be generated during thermal decomposition of the environmental matrix and chlorine-containing substances that may be present in it. To ensure selectivity an extraction method for PVC *via* solvent extraction has been developed. In the first step methanol is used to eliminate chlorine-containing substances such as PCBs. In a second step using tetrahydrofuran (THF) PVC is extracted and separated from inorganic compounds such as sodium chloride. The THF extract is sorbed onto silica gel and transferred into the combustion system. Validation of the influence of chlorine-containing substances and organic components revealed no disturbance of PVC determination by these compounds. So the presented method is suitable for a sensitive and selective determination of PVC in complex environmental samples.

3.09.P-Mo177 Separation and Purification Method for Microplastic Analysis of Glacimarine Sediment (Disko Bay, Greenland)

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Separation and purification methods for microplastic (MP) analysis are greatly dependent on the type of matrix and size fraction of target MP particles. In general, MP extraction methods for sediments commonly include in-between steps such as sample transferring and intermediate filtrations for the removal of biogenic and inorganic matter. However, all those intermediary steps increase the sample processing time and pose a risk of MP loss, especially for the small size fraction (< 100 µm). This work proposes a four-step separation and purification method for MPs in glacimarine sediments (Disko Bay, Greenland), where all purification steps are carried out in one single glass flask, transferring the sample only once for the density separation to minimize particle loss. The sample was initially dried and treated with 10% acetic acid to remove foraminifera shells. The digestion procedure was performed with 5% KOH and 7% NaOCl, which was neutralized prior to density separation (ZnCl₂ 1.6 g.cm⁻³) to avoid precipitation. Each step required a 24h resting and settling time. All reagents have demonstrated not to damage the MPs. The method efficiency and recovery were evaluated spiking PE fluorescent microspheres (63-75 µm, 125-150 µm and 300-500 µm) in the sediments. All analyses were carried in triplicate and the density separation was performed twice per sample for efficiency evaluation. The floating fraction was filtered with a customized metal filtration system using steel filters with mesh size 10 µm and 100 µm. This system allows backflushing the MPs directly onto a membrane for further chemical characterization. The average recovery was higher than 90% for particles >100 µm and 82% for particles >10 µm. The majority of the large particles were collected in the first density separation, whereas 50% of the collected particles >63 µm were extracted in the second separation. The method was also compared to performing intermediate filtrations and sample transferrings showing 90% recovery particles >100 µm and 72% for particles >63 µm. This demonstrates that the separation and purification processes that minimize intermediate steps in this method, improve MP recovery and assessment accuracy. Furthermore, this method can be applied to

different type of sediment samples where the expected amount of MP is low, such as glaciamarine sediment and samples from more pristine areas.

3.09.P-Mo178 Assessment of Oxidative Digestion Protocols Effects on the Integrity of Virgin and Aged Microplastics Through Scanning Electron Microscopy Analysis

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Microplastics (MPs) presence in the environment is a certainty by now; therefore its monitoring is fundamental to assess their potential risk for human health and ecosystems. Despite these assumptions, there are still huge gaps and uncertainties in the validation of analytical methodologies. Preliminary purification of samples is required before MPs quantification to isolate them from the environmental matrix. Usually, chemical digestion treatments based on oxidizing reagents (acids, bases, enzymes) are used to remove the organic substance facilitating particles extraction. However, some of these are aggressive with a risk of damaging particles and underestimating data. Moreover, most of the methods assess digestion treatments on virgin MPs ignoring that their impact on environmental MPs could be higher due to the fragility of these particles already exposed to environmental conditions. In this context, our study aims to evaluate the impact of the most common oxidative treatment (Fenton's reaction) on virgin and aged MPs of different shapes and sizes. Six polymers (polyethylene, polypropylene, polyamide, polystyrene, polyvinylchloride, and polyethylene terephthalate), were selected among the most used plastic items. MPs standards were artificially aged in a climate room and changes in chemical bond structures were measured by Fourier Transform Infrared spectroscopy analysis. Different digestion treatment conditions were evaluated changing temperature (75, 50, 30°C) and reagents volume (100, 60mL H₂O₂) on virgin and aged MPs. Particles integrity was observed by *Scanning Electron Microscopy*, before and after treatments. The overall results showed morphological and dimensional alteration of all particles of different entities. On the one hand, virgin MPs exhibited evident cracks, holes, corrosion in polyamide, polyvinyl chloride, polypropylene, and polystyrene caused by the treatment at 75 °C. The protocol at 50 °C impacted just polyvinyl chloride and polystyrene particles, while at 30 °C generated a dimensional reduction and corrosion of the margins of polyethylene and polypropylene. On the other hand, the stronger treatment caused the loss of material and the damage of all aged particles and pellets abrasions. In contrast to virgin MPs, the milder and intermediate treatment did not produce strong changes. In conclusion, the temperature may influence the integrity of the particles and this may alter the quality of the final data.

3.09.P-Mo179 Comprehensive Analysis of Plastics in Vulture Regurgitates: From Mega to Micro Size

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Landfills are important sources of plastic pollution in the terrestrial environment, and this is of special concern for opportunistic species foraging in these sites. Vultures are obligate scavengers and frequently use landfills as important alternate food resources. Previous studies have reported plastic consumption in vultures, but they have focused on large plastic particles disregarding small microplastics (sMP, < 1 mm size). In this study, we evaluated occurrence of large plastic particles, MP and sMP in a Cinereous vulture (*Aegypius monachus*) population in Sierra de Guadarrama, Madrid, Spain, using regurgitates as a proxy of ingestion. Eighty-eight nests were visited during the breeding season in 2020 from a population breeding close to a landfill. The regurgitates and plastic material present in regurgitations were collected from each nest (n = 88) and the surrounding area (15 m radius from nest tree) and stored in paper envelopes. Once at the lab, the regurgitates material were weighted and visible plastic particles were separated. These plastic particles were sorted in two different groups for further qualitative and quantitative analyses: mega-plastic (> 100 mm) and macro-plastic (> 20 – 100 mm). The color, area, size, and number of particles were evaluated, and the polymer type identified by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR). The remaining regurgitate material for 55 samples was digested for 48h using KOH treatment and sieved into meso-plastic (> 5 – 20 mm), MP (> 1 – 5 mm) and sMP (< 1 mm). A novel framework based on infrared hyperspectral imaging (IR-HIS) was then used to detect and quantify meso-plastic, MP and sMP by polymer type and size down to 150 μm. Our study is the first to provide such specific data on plastic regurgitates, down to micrometer size, in a terrestrial raptor species.

3.09.P-Mo180 Can Microplastics in Molluscs to Have a Potential Risks for Human Health of Catalonia Coast Habitants?

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The determination of an ingestion rate is essential to assess potential human health risks of microplastic ingestion but due the consumption rates varies widely by country, culture, lifestyle options, and geography, the ingestion rate must be estimated according specific zones conditions and features. Because Catalonia is a great producer and consumer of molluscs, this study focused on microplastics (MPs) concentrations estimations of commercially relevant molluscs from Catalan coast (northwest Mediterranean Sea) and MPs exposure assessment in Catalan people as a first step for an estimate of high confidence on microplastics exposures by seafood quantity intakes in Spain. An accurate and practical methodology for MPs extraction and identification was applied to high quantity of seafood samples (50-70 g), improving existent previous methodologies as a contribution for standardized protocols for analysis of MPs in biota. Seafood samples depurated or not were collected along Catalonia Coast (NE Spain) in October-November 2017. Seafood samples included wedge clams (*Donax trunculus*), marine snail (*Bolinus brandaris*), razor clams (*Ensis silicua*), fine clams (*Tapes decussatus*), big oyster (*Crassostrea gigas*) and musells

(*Mytilus galloprovincialis*) from north, middle, and south zone at depurate and not depurated conditions. The dietary intake of MPs from depurated mussels, oysters and clams of Catalan population was estimated by applying the following equation: $E_t = C_f * X_{t,f}$, where E_t is the dietary exposure to the MPs (MPs/day), C_f is the mean consumption of that molluscs (f) (g/day) by population and $X_{t,f}$ is the concentration of the MPs in each samples group f (MPs/g wet weight fresh and soft tissue). The mean consumption of each age group was after normalized by dividing the dietary intake by the mean body weight of each age group. An optimized method for analysing MPs in molluscs samples has been developed successfully for reliable MPs quantification. The average MPs concentration values in molluscs found in this study were higher than reported for previous studies, vulnerable population as adults 40-65 years (higher incidence of chronic diseases) and pregnant woman have high MPs diary intakes compared with the intakes of entire population from other countries. A systematic and comprehensive analysis of MPs concentration in molluscs from Catalan coast and local consumption seafood surveys are needed to clarify the status of human exposure of Catalan people

3.09.P-Mo181 Plastic and Non-Plastic Microparticles Show Surface-Related Effects on the Reproduction of the Nematode *Caenorhabditis elegans*

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Microplastic particles can disturb the food availability of nematodes and, by this indirect effect mechanism, also inhibit their reproductive output. Thus, microplastic might pose a risk to aquatic organisms under realistic conditions, where food availability is not always guaranteed. Benthic invertebrates, such as nematodes, live and feed in sediments, consisting of organic and inorganic particles of all sizes and shapes. Therefore, any observed effects of synthetic plastic particles on benthic invertebrates must be put in perspective to the abundance of natural particles in their habitat, for evaluating the ecological risk of microplastic. We tested a variety of microplastic particles of various sizes (0.1 to >100 µm), shapes (beads, fibers, fragments) and polymers (PS, PFTE, PET, PA) in terms of their effects on the reproduction of *C. elegans* using a standardized test protocol (ISO 10872) and compared the effects to those of naturally occurring microparticles, such as clay minerals (dolomite, kaolin), silica beads and plant spores. We could show that, independent of the material or polymer, the particle size determined the quantity of the nematode's response (i.e. EC50). However, if relating the effect to the total surface area of the particles, a size-independent EC50 (expressed as cm²/ml) could be identified for beads and fragments. These results bring up several questions that should be discussed in context of the effect and risk assessment of microplastics in the environment: (1) Do microplastics add an additional risk to organisms that live in particle-rich environments, such as soils and sediments? (2) Can naturally occurring particles, such as minerals or spores can serve as reference particles in routine toxicity testing of microplastics? (3) Is the total surface area of particles a suitable parameter to predict adverse effects of microparticles?

3.09.P-Mo182 In-Vitro Assessment of the Sensitivity of Bivalve Hemocytes to Nanoplastic Exposure

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Plastic particles are prevalent in the environment, and the risks they pose especially to aquatic organisms are of growing concern. A range of responses attributed to plastic particle exposure have been documented across various levels of biological organization, extending from cellular to population-level endpoints. However, systematic analyses of sub-lethal endpoints at the cellular level, which are considered as a sensitive response variable to plastic particle exposure, are still lacking. It has been established that plastic particles can be internally distributed in organisms through various ways, such as translocation and penetration of biological barriers. In particular, particles with dimensions in the order of nanometers (i.e. nanoplastics, NPs, < 1000 nm) are able to enter cells via endocytotic pathways (i.e. phagocytosis). These cellular processes have been highlighted as integral factors that influence key physiological functions including cellular immunity. Hemocytes are responsible for cell-mediated immunity in bivalve mollusks such as the mussel *Mytilus edulis*, which is highly susceptible to NP exposure due to its sedentary and filter-feeding life style. The hemocytes circulate within the hemolymph and can cross all epithelial boundaries, acting as phagocytes against foreign particles, including plastic particles. Assessing the different physicochemical properties of NPs, such as polymer type, size, shape, and surface charge, is crucial as these parameters may directly affect the cellular uptake mechanisms as well as cytotoxicity. Hence, this study aims to mechanistically evaluate whether NPs induce cytotoxicity in the different hemocyte subpopulations of mussels *M. edulis*. The timescale and extent of cellular responses will be assessed, particularly those related to apoptosis and the production of reactive oxygen species (ROS) as a biomarker for oxidative stress. An in-vitro approach will be applied to characterize and interpret the response profiles under environmentally relevant exposure conditions and in assessing differential effects linked to characteristics of the plastic particles (type, size, shape). The assessment of the cellular responses to plastic exposure will be carried out through the complementary use of flow cytometry and fluorescence microscopy. The results will provide insights into the underlying mechanisms associated with different physicochemical characteristics of NPs at a cellular level which is crucial for assessing their fate and toxicity in aquatic organisms.

3.09.P-Mo183 Ingestion and Toxicity of Polystyrene Nanoparticles (PS-NPs) on Artemia larvae (Artemia urmiana)

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Currently little knowledge is available on the impact of nanoplastics (defined as being < 1 or < 0.1 micron depending on the definition used) on marine organisms. Although the amount of nanoplastics in the aquatic environment is not yet known, it is expected that nanoplastics will have a different impact on aquatic organisms than larger microplastics due to their small size, high surface curvature, and large surface area. The current study aimed to evaluate the effects of 40 nm anionic carboxylated (PS-

COOH⁻) and 50 nm cationic amino (PS-NH⁺₂) polystyrene nanoplastics (PS-NPs) on brine shrimp *Artemia urmiana* larvae. Larvae were exposed for 14 days with a concentration of 0.5, 1, 1.5, 2.5 and 5 ppm and then depurated for 24 h in clean medium. Measured endpoints included mortality, growth, total fatty acid and protein content. For both PS-NPs (5–100 ppm), nanoplastics were found in the gut lumen of larvae (24 and 48 h) and adsorbed at the surface of sensorial antennules and appendages. Some of PS-NPs were excreted as fecal pellets following 24h depuration in clean seawater, but a complete emptying of the gut was not observed. Larvae exposed to PS-NH⁺₂ underwent multiple molting events during 24 and 48 h of exposure compared to controls and PS-COOH⁻. No significant *Artemia nauplii* mortality was found following 48 h of exposure for both PS-NPs in the tested concentration range (5-100 ppm). Significant mortality was observed after 14 days of exposure for both PS-NPs of different concentrations (0.5-5 ppm) at nauplius stage. The results also showed that exposure of *Artemia urmiana* larvae to anionic carboxylated (PS-COOH⁻) after 14 days reduced total fatty acid and protein concentrations by 15.4% and 9.4%, respectively. Nano-sized PS was shown to impair feeding and motility, and cause multiple molts of brine shrimp larvae indicating that the sub-lethal effects of nanoplastics should be further investigated

3.09.P-Mo184 Effect-Directed Analysis (EDA) of Beach Plastic (PlastEDA) From Baltic Sea

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The rising presence of plastic debris along with chemical pollution in aquatic environments caused increasing concern regarding potential impacts on human and environmental health. Apart from physical effects, plastic particles contain toxic additives, and these particles adsorb various chemicals present in the surrounding environment. Upon reaching in the living organisms' gut these chemicals may desorb which lead to adverse effects. Beach plastic was collected from Gotska Sandön, an island in the Baltic Sea, south-east of the Swedish mainland. The samples were cleaned, and polymer type characterization was conducted using a fourier transform infrared (FTIR) spectroscopy. Each polymer was grinded separately and sieved into various sizes by using steel sieves. For size characterization a Multisizer Coulter counter III (MSIII) was used. Each particle size group was extracted separately with a mixture of organic solvents. For the measurement of the mechanism-specific hazard potential, the plastic extracts were screened with different Chemical Activated Luciferase gene eXpression (CALUX) bioassays for agonistic and antagonistic activities for various nuclear hormone receptors (estrogenic, androgenic, and thyroid), aryl hydrocarbon receptor (AhR) and for mutagenic response i.e., P53. Complexity of samples is reduced by using gas chromatographic- (GC-) fractionation with parallel mass spectrometric (MS) detection system and obtained fractions are biologically analyzed. Bioactive fractions are chemically screened by using quadrupole-time of flight mass spectrometry linked to gas chromatography (GC-QTOFMS). Preliminary results indicate high estrogenic and AhR potencies in various extracts, which were selected for effect-directed analysis (EDA) to identify chemicals that are causing these effects.

3.09.P-Mo185 Sea Urchin Interaction With Nano-And Microplastics Particles: Size and Morphology Influence on Ingestion-Egestion

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Microplastics on sea bottom have potential to be ingested by benthic organisms with adverse effects as disrupting feeding and digestion, energy reserves and fecundity decrease. In addition, hazardous substances released from microplastics might impact these organisms; this implies that microplastics accumulation and interaction time in organisms could be directly related to their toxic effects. In this study, adult and larvae sea urchins (*Paracentrotus lividus*), a common Mediterranean benthic organism, were used to study ingestion, egestion and body interactions with micro and nanoplastics. Several types of plastics were used: polyethylene (PE) spheres of 53-63, 125-150, 250-300 and 425-500 µm of diameter, polyamide (PA) fibres and polystyrene (PS) nanoplastics (80 nm of diameter). For adults, six experiments were designed with microplastics at same weight ratio (1:1): ingestion 48 h, ingestion-egestion 48 h, ingestion 96 h, all of them at two concentrations 50 and 250 mg/L. For larvae (pluteus-4 arms), were tested four concentration of 53-63 µm PE spheres and fibres (1.4 mg/L, 2.5 mg/L, 12 mg/L, 26 mg/L) and 80 nm PS nanoplastics (0.05 mg/L, 0.26 mg/L, 2.6 mg/L, 26 mg/L) in 24 h. Results showed that adult sea urchins actively ingested PE spheres of 53-63µm and 125-150 µm, conversely, 450-500 µm the biggest PE spheres and fibres were the less ingested, although the fibers showed signs of chewing. For 53-63 µm, adults sea urchin ingested 1285 ± 1056 per individual (0.2 % of particles added) and egested up to 84 % of the quantity ingested. Regarding 125-150 µm PE spheres, 267 ± 244 were ingested per individual (0.5 % of particles added) and egested up to 82 % of the quantity ingested. The total body interaction (plastic particles present in intestine, plates, spines, tube feet and water vascular system) with these both particles was 0.6 % of the total particles added. The ingestion-96 h rate was similar to ingestion-48 h for 250 mg/L test and for all sizes and morphology with r²= 0.82. The adults does not select the particles, they feed depending on particles concentration and distribution, the spheres were more easily ingested, the microplastics were not fully excreted within 48 hours, the ingestion rate 48 h was similar to ingestion 96 h. Regarding larvae, microplastics concentration 26 mg/L and nanoplastics concentration 2.6 mg/L decreased the 24 h-survival rate to 74 % and 78%, respectively

3.09.P-Mo186 Toxic Effects of Micro- and Nanoplastic (MNP) Across Different Taxonomic Groups and Their Key Mechanisms

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Detrimental biological effects of micro- and nanoplastics (MNPs) have been observed on a variety of organisms. A fast-growing number of toxicological studies report diverse responses and wide species-dependent sensitivity upon MNP exposure. While studies are dominated by *in vivo* animal tests, our understanding of cellular toxicity and the corresponding toxicity mechanisms is still limited. This challenges the proper assessment of environmental hazards and health risks of MNPs. In this presentation, we gathered and analyzed recent studies including our own laboratory data on human cells, animals, plants, alga, and bacteria, and identified the similarities and differences in toxic effects and key mechanisms of MNPs across different taxonomic groups. In general, toxicities are often observed at high doses (~mg/L) and the effects and mechanisms of MNPs depend on their sizes, surface characteristics, polymer types, as well as cell types. Plausible toxicity mechanisms mainly include membrane disruption, extracellular polymeric substance disruption, reactive oxygen species generation, DNA damage, cell pore blockage, lysosome destabilization, and mitochondrial depolarization. The identified plausible toxicity mechanisms are useful to prioritize and improve future *in vivo* and *in vitro* toxicological and risk assessment studies of MNPs.

3.09.P-Mo187 The Toxicity of Microplastic Degradation Products to PLANTS Following Composting

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Microplastics are small pieces of plastic that pollute the environment. Microplastics are not a specific type of plastic, but rather any plastic fragment that is less than 5 mm in size. They enter the environment via many sources, including cosmetics, clothing, and industrial processes. Once in the oceans, microplastics can either float or sink. Plastics degrade slowly, over hundreds or thousands of years. This increases the likelihood of microplastics being ingested and incorporated into, and accumulated in, the bodies and tissues of many organisms. This may have toxic effect on the organisms. Or the microplastics may enter the food chain, especially via fish and shellfish. This could have consequences for the health of human consumers. Variations to existing standardised biodegradation methods or potentially entirely new standardised testing methods are likely to be necessary to appropriately assess the (bio)degradability of microplastics in the environment. However, application of existing standardised methods can provide valuable information on the (bio)degradability potential of some microplastics. Unfortunately, unlike standard biodegradable chemicals, very little is understood about the environmental impacts of degraded microplastic material. These are either disintegrated into smaller micro or nano plastics or into their composite monomers. The toxicity profiles of these degradation products might not be known. Here we present our early results from a successive composting test on a series of micro plastic materials culminating in a plant toxicity tests to determine the toxicity of the degradation products.

3.09.P-Mo188 Plastic Contamination of Edible Parts of Marine Organism in the North Atlantic French Coast

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For the last decades, scientific community agreed on the widespread of microplastics (MPs, < 5 mm) in aquatic environments. MPs are ingested by most of marine organisms, including the ones consumed by humans. In this study, we evaluated the MPs contamination of the aquatic biota and determined the exposure via seafood consumption, both for French consumers as well as world average eater (Arplastic project). The present study aimed at describing the MPs contamination in the aquatic foodweb of Arcachon Bay (France) along the year 2019 by selecting four edible species as pacific oyster (*Crassostrea gigas*), sea spider crab (*Maja squinado*) and two fish, a benthic one (common sole, *Solea solea*) and a pelagic one (sea bass, *Dicentrarchus labrax* or *punctatus*). All sorted particles detected in organisms were chemically characterized by ATR-FTIR. Among all particles, fibers was the predominant shape for all species (75-100%) and color depended on species and were mainly blue, white or black. Cellulosic particles ($1836 \pm 1321 \mu\text{m}$) represented 69 to 84%, equivalent to 1.60 ± 1.74 items/individual for *M.squinado*, while other species contained in average 0.62 ± 1.15 items/ind. Besides, MPs contamination ($2022 \pm 1923 \mu\text{m}$) represented less than 1 particle/individual in average, corresponding to 0.0083 ± 0.023 MPs/g (ww). However, particles contamination did not vary over time, and no seasonal trend was observed. Particle's contamination in organisms collected in Arcachon Bay participated to assess the potential seafood contamination of humans. According to the results of this study, we can estimate that a medium French seafood eater would ingest in average 4 to 5 cellulosic particles per week depending if it is a men, a women or an elderly, while they would consume 1 MPs per week. More globally, a world average eater would ingest 6 cellulosic particles and 2 MPs per week. This work contributes to a better consideration of MPs in environmental and health risk assessment.

3.09.P-Mo189 Embryotoxic and Neurobehavioral Effects of Different NanoPlastics on the Early LIFE Stage in Zebrafish (*Danio rerio*)

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Abstract: Nanoplastic has attracted global attention due to its potential ecological and health impacts. Current ecotoxicological studies on animals are dominated by using plain polystyrene nanobeads, however, our understanding of the toxic effects of other types of nanoplastics is left far behind. In this study, we compared the embryonic and neurobehavioral effects of different nanoplastic types, including polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET), and polyethylene (PE), polyester-polyurethane (PUR), polycaprolactone (PCL), and polyamide (PA). Early-life stage zebrafish embryos and larvae (2 to 120 h postfertilization) were used as the model. The impacts of nanoplastic exposure on hatchability, embryonic development rate, mortality, and deformity rate, and brain histology were measured. Furthermore, the larval locomotor activity, as a highly sensitive and rapid neurobehavioral indicator, was quantified and compared between different nanoplastic treatment and control

groups. These comprehensive results provide new insights into the similarity and difference in toxicity of different types of nanoplastics and highlight the challenge of assessing the ecological risks of nanoplastics due to the great complexity of nanoplastic mixtures in the environment.

3.09.P-Mo190 Maternal Exposure to Plastics Impacts Fetal Development in a MOUSE Model of Pregnancy

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Plastics are ubiquitous in modern society and when released into the environment, they break down into smaller particles termed microplastics (plastic particles with diameters < 5mm) and nanoplastics (diameter < 1 um). These micro- and nanoplastics can be ingested by organisms and potentially accumulate in tissues and organs. Humans are primarily exposed to plastic particles by inhalation of dust, ingestion of food contaminated by dust fall, and through drinking water. Recently, 5-10 um plastics were found in the placentas of healthy women, raising the concern that plastics exposure may have an impact on pregnancy and fetal development. In this study, we investigated the effect of maternal exposure to plastics on fetal growth and brain development using experimental mice. The mouse reproduces many of the physiological and molecular features of pregnancy, making it a commonly used model to study pregnancy. Throughout gestation (gestational day 0.5 to 17.5; full-term is 18.5), pregnant CD-1 dams were exposed to 5 um polystyrene plastics in filtered drinking water at concentrations ranging from 0-1000 ug/L (n=11-12 dams/group). The pregnant dams were sacrificed at E17.5 and fetal and placental weights were recorded. The umbilical cord length was also measured. In a separate cohort, dams were sacrificed at gestational day 15.5 and fixed for scanning by X-ray micro-computed tomography. 3D datasets were acquired and compared between groups to assess differences in embryo morphology. While there was no significant difference in placental weights between groups, the fetal weights were significantly decreased with plastics exposure in a dose-dependent manner (p=0.002). There was also a significant decrease in the umbilical cord length (p< 0.0001), suggesting decreased fetal growth and decreased fetal movement. We will also present the results of the micro-computed tomography study to detect anatomical differences between groups. This study demonstrates that maternal exposure to plastics at a high concentration have a deleterious effect on placental and fetal development.

3.09.P-Mo191 Does Exposure to NanoPlastics Impact Pregnancy Outcome and Fetal Development?

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There is growing concern about the potential human health impacts of exposure to nanoplastics (diameter < 1um). Humans are primarily exposed to nanoplastics by inhalation of indoor dust and ingestion of food contaminated by dust fall. Following exposure, nanoplastics are small enough to translocate from the lungs into the blood stream and accumulate in other organs. A recent study reported plastics (diameter 5-10 um) in the placentas of health women, raising the concern that exposure to plastics may have an impact on pregnancy and fetal development. In this study, we investigated the effect of maternal exposure to nanoplastics on fetal growth and postnatal brain development using experimental mice. The mouse is a well-accepted model of human pregnancy, reproducing many of the physiological and molecular features of pregnancy. There is also considerable cross-species alignment between mouse and human fetal neurodevelopment, making the mouse a good model for studying brain maturation. Throughout gestation (18.5 days) and during lactation (23 days), pregnant CD-1 dams were exposed to 50 nm polystyrene nanoplastics in filtered drinking water (1000 ug/L, n=5 dams). A control group drinking filtered water without plastics was also included (n=5 dams). During the neonatal period, the pups were weighed weekly and underwent a series of behavioural tests to determine the timing of developmental milestones including righting reflex (from postnatal day 2-6) and eye opening (from postnatal day 9-17). At weaning (postnatal day 23, equivalent to adolescence in a human), a subset of the pups (2 females and 2 males/litter) were sacrificed by perfusion fixation for ex vivo high-resolution 3D magnetic resonance imaging (MRI) to investigate neuroanatomical differences between the groups. A second subset of pups (2 females and 2 males/litter) were sacrificed for metabolomics studies of the brain tissue using high-resolution magic angle spinning magnetic resonance spectroscopy. The MRI and metabolomics data will be correlated with the outcomes from the behavioural tests. This study demonstrates the impact of maternal exposure to nanoplastics on pregnancy outcome and fetal development.

3.09.P-Mo192 Endocrine Disruptive Effects of Disposable Face Mask Leachates on the Androgen Receptor Within an Aqueous Environment

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To fight the spread of Covid-19, the use of disposable face masks increased drastically as most countries made it compulsory to wear a mask in public. The face masks are made up of polymeric materials, mainly polypropylene. Some well-known leachates from plastics are phthalates, bisphenol A (BPA) and nonylphenols, which are known to cause endocrine disruption. Since masks are not being discharged as medical waste only they end up in landfills, and due to weather conditions end up in water bodies. This study aimed to determine whether leachates from face masks have (ant)agonistic effects towards the androgen receptor (AR) using the MDA-kb2 reporter gene assay. Face masks were leached in ethanol for 24 and 48 h at 4°C and 30°C to allow the leaching of water-soluble compounds. The MDA-kb2 cells were exposed to the leachates and because these cells are genetically modified with a transcribed luciferase enzyme, (ant)agonistic AR effects could be determined by measuring the luminescence produced. The glucocorticoid receptor (GR) is also under the control of the same reporter gene construct in the same cell line. The amount of light emitted by the cells is expressed in terms of the known reference compound: testosterone (T) for the AR and

dexamethasone for the GR. For antagonism, the cells received T during seeding and the known antagonist, flutamide, was used as reference compound. The MTT viability assay was also performed to determine the cytotoxicity of the leachates but no cytotoxicity was recorded. Neither dose-related agonistic effect of the AR or GR was observed, nor quantifiable antagonism of the AR at the concentrations tested. Since the face mask leachates did not show any effect on the AR and are considered non-cytotoxic, it is possible that the leaching of toxic compounds did not occur or that the concentrations of the leachates used are not sufficient for quantification of endocrine disruptive activities. Many variables from the study can further be experimented with for example extended leaching periods, adjustments to leaching temperatures, use of different leaching solvents and exposing the cells to different leachates concentrations. Further research on mask leachates is crucial to protect human and environmental health from this emerging pollutant.

3.09.P-Mo284 Paint Particles in the Weddell Sea (Antarctica): A Forensic Investigation

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Microplastics (MP; < 5mm) are ubiquitous in the marine environment and considered as an emerging threat to marine biota what raised extensive scientific attention over the recent decades. Paint particles, which have similar compositions to microplastics, however, received little attention, even though antifouling-paints can have more severe impacts on marine biota than other synthetic particles. In this study, we evaluate MP (>300 µm) concentration and composition in surface (n=34) and subsurface water samples (n=79, 11.2 m depth) of the Weddell Sea. All putative MP were analyzed by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. MP was found in 65% of surface and 11.4% of subsurface samples, with mean concentrations of 0.01 (±0.01 SD) MP m⁻³ and 0.04 (±0.1 SD) MP m⁻³, respectively, being within the range of previously reported values for regions south of the Polar Front. Of all recovered MP, 47% are possibly associated to ship paints due to their polymer composition, visual characteristics and brittleness. This indicates that paint particles might be an important microplastic source in the southern ocean. Additionally, particles with same colors as paints from RV *Polarstern* were found (n=394) but could mostly not be assigned to a polymer by means of FTIR due to low quality matches with the spectral library. Particles of that type (n=101) with similar FTIR spectra to reference paints from the research vessel and fresh paint references generated in the laboratory, were further subjected to micro-X-ray fluorescence spectroscopy (µXRF), a method also used in forensic science to discriminate paint particles left on traffic accident sites based on the composition of inorganic pigments and additives. Besides Ti, Ca and Fe, which were found in nearly all particles, heavy metals such as Cu, Pb and Zn were detected. Further µXRF measurements revealed that 45.5% of all recovered synthetic particles derived from vessel-induced contamination, while 11% of the measured paint fragments could be distinguished from the reference paints via their elemental composition and might derive from other sources. This study demonstrates that low-quality matches for FTIR spectra of ship paints might lead to underestimation of paint particles in the marine environment if not analyzed further. The additional evaluation of inorganic pigments and additives is needed, to evaluate and understand the full contribution of paint particles to the marine MP load.

3.10 Microplastics in the environment: Behaviour, transport, fate, risks, and alternatives to conventional plastics (Part I)

3.10.T-01 Biodegradation of Engineering Plastics: Influence of the Polymer Backbone on TPU Microplastic Degradation and Fragmentation

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Since plastic pollution increased over the last decades, authorities had to take actions: Bans, reporting and labelling requirements for intentionally added primary microplastics serve to avoid their release into the environment during or after product application. Biodegradable polymers are derogated from the restriction proposed by the European Chemicals Agency, thus putting a lot of focus on the development of biodegradable alternatives. Biodegradation is based on microbial oxidation and hydrolysis processes in environmental matrices that result in the complete transformation of polymer carbon into biomass and CO₂. Polymers based on polylactic acid and polybutylene adipate terephthalate are commonly used biodegradable plastic products, which is why its biodegradation processes have been intensively investigated. In contrast, little is known about biodegradation of engineering plastics like thermoplastic polyurethanes (TPU). TPUs based on polyester polyols provide the possibility for hydrolysis, which might make them susceptible to microbial attack. Here we studied biodegradation of TPU esters in compost, and systematically varied the polymer backbone to provide guidance to future polymer development. We investigated the influence of a hydrolysis stabilizer, low and high hard segment contents and crosslinking on the fragmentation and degradation of TPU microplastics. During biodegradation (ISO 14855, part of EN13432) CO₂ evolution was monitored. To gain insights into the fragmentation, we developed and validated a non-destructive and efficient extraction protocol for the partially biodegraded TPUs from compost. We assessed the particle number and the particle size distribution via fluorescence microscopy (staining with Nile Red) and FT-IR microscopy. In addition, we confirmed the reduction of molar masses by GPC and the hypothesized increase of crystallinity by WAXS. We successfully developed methods to extract TPUs from compost and to monitor biodegradation of TPUs in compost. Our research revealed that hydrolysis stabilizers change the rate and pathway of fragmentation, that a low hard phase content increases biodegradability and that crosslinking reduces biodegradability. These are safer-by-design principles for the development of alternatives or improved versions of conventional plastics.

3.10.T-02 Impacts of Polyethylene Microspheres on the Freshwater Duckweed *Lemna minor*

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There is great concern regarding the abundance and impacts of plastics, namely microplastics (particles < 5 mm), in aquatic ecosystems. Microplastics can derive from a variety of sources, reach high densities, adsorb chemical compounds from water/sediment compartments and cause negative impacts on the environment and in human health. This issue has been well documented for marine environment; however, freshwater systems are still overlooked leading to a gap in knowledge, especially about the impacts of these micro-sized particles on primary producers. Hence, this is a pioneering study aiming to evaluate the impacts of polyethylene microspheres on the freshwater duckweed *Lemna minor* by assessing the ecotoxicological effects on the growth, root length, and nutritional profile (i.e., fatty acids and carbohydrates composition and abundance). Results pointed out that growth (based on fronds number and dry weight), root length and the nutritional profile were not affected by polyethylene microspheres. In fact, when exposed to the highest concentration the growth rate and yield of *L. minor* were significantly stimulated. Regarding the root length, it was observed an inhibition trend that does not follow a dose-response relationship. The composition and abundance on fatty acids and carbohydrates were not significantly affected by polyethylene microspheres guaranteeing the important input of non-synthesized fatty acids (e.g., essential fatty acids – omega-6 and omega-3) and carbohydrates into higher trophic levels. Moreover, polyunsaturated fatty acids and rhamnose increased their abundance over the range of polyethylene concentrations suggesting that they were the main contributors to *L. minor* resisted polyethylene exposure. Thus, polyethylene microspheres at environmentally relevant and higher concentrations do not present a hazard to freshwater macrophytes.

3.10.T-03 From Rivers to the Tap: Investigation of Micro- and NanoPlastics in Drinking WATER by Pyrolysis-Gc-Ms

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The occurrence of micro- and nanoplastics (MNPs) in freshwater has been globally recognized. This raised the question of possible human exposure to MNPs through drinking water from freshwater sources. Recent studies have shown that potable water may contain MNPs, but a high degree of variation in concentrations has been reported. These variations are attributed to the differences between raw water and the treatment and distribution processes. However, the analytical techniques employed for the identification and quantification of MNPs also have a significant role at the level of reported. To be able to estimate the MNP associated risks via drinking water consumption, reliable exposure concentrations with lower size limits of polymers are urgently needed. In the present study, a sensitive method was developed to detect and quantify MNPs in water samples based on the protein corona mediated aggregation of particles followed by filtration and analysis with pyrolysis-gas chromatograph-mass spectrometry (Py-GC-MS). The method was validated using different types of water and acceptable recoveries were obtained for PE, PP, PVC and PS ranging from 97 to 118%. Given the ubiquity of microplastics in freshwater systems and increased particle numbers with decreasing polymer sizes, this method is a promising tool to quantify MNPs in water samples and to evaluate the risks for human health.

3.10.T-04 A Sequential Extraction Scheme to Selectively Analyze Adsorbed (Trace) Elements on Plastic Particles

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Plastic pollution is an environmental issue of concern, as the mismanagement of plastic wastes strongly impacts water environments. Plastic particles were recently observed to adsorb dissolved (trace) elements in water environments, and this issue has attracted interest of researchers in order to understand their interaction mechanisms. Accordingly, the analysis of plastic environmental samples plays a crucial role in understanding the likelihood of such interactions. Recent approaches focused on the total extraction of metals from plastic samples through acid digestion, which dissolve the polymer matrix and overestimate the elemental load of plastics. In addition, this procedure does not provide any information on the speciation of the adsorbed elements: the implementation of a detailed speciation scheme is therefore strongly advised. In this study, we propose a three-step extraction protocol that was tested on different plastic particles sampled from a freshwater environment. Plastic samples were obtained grinding macroscopic plastic materials collected on the shores of an oligotrophic lake (Lake Como, Italy). Non degraded plastic was also investigated to assess the effect of degradation on the metal adsorption capacity. Sequential extraction was performed using empty solid phase extraction cartridges with the following reagents: i) ammonium nitrate 1M, to extract physisorbed metals; ii) acetic acid 0.1M to extract metals in slightly acid condition; iii) hydrogen peroxide 30% to extract metals bound to organic matter and biofilms. Solutions were then analyzed by inductively coupled plasma – mass spectrometry. Kinetics test in the 1-72h range suggested that equilibrium in the extraction was achieved after 24h under shaking. The comparison of environmental and unaged samples showed that the former generally enrich in different micronutrients (Fe, Sr and Mn) and other trace elements (Sn, Pb, Ba and partly Ti), which show concentration at least ten-fold higher than in the unweathered material. Moreover, most of the microelements were prevalently present in the H₂O₂ extraction step, indicating organic species as the prevalent form of the elements onto plastic surfaces and pointing to biofouling as the major mechanism for (trace) elements

accumulation onto plastics from lake water. These results will be then compared with different single-extraction protocols present in the literature and applied to microplastic samples in different water environments.

3.10 Microplastics in the environment: Behaviour, transport, fate, risks, and alternatives to conventional plastics (Part II)

3.10.T-06 Microplastic in Sediment and Macroinvertebrates From a Norwegian Stream in Connection to a Plastic Recycling Plant

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Freshwater systems are close to sources of microplastics from e.g. sewage treatment plants, runoff from urban and agricultural areas and industry. e.g. plastic recycling plants. Freshwater systems have been found to not only act as a vector for transport of microplastics to the marine environment but also to accumulate microplastics in the sediment. Benthic macroinvertebrates are important component of freshwater ecosystems and expected to be exposed to microplastic as they live in close association with the sediment. Macroinvertebrates has been widely used to assess the status of a freshwater system, as the very diverse groups shows tolerance/sensitivity towards different stressors, and some of them have long life cycles which enables accumulation of contaminants. Apart from monitoring, macroinvertebrates also make up an essential link to the higher trophic levels in the freshwater food web and might thus constitute an essential pathway for microplastic into the food web and if ingested, microplastic might act as a potential stressor in these organisms. Despite the obvious exposure risk, it is still unknown how and if this macroinvertebrates can be used as a sentinel for microplastic pollution as there is a lack of studies on freshwater macroinvertebrates, and little is known about the occurrence of microplastic, the correlation with microplastic in the sediment and the effect from feeding trait and lifestyle. This study focusses on plastic in three macroinvertebrates species living in a natural environment, however heavily polluted by microplastic due discharge from a plastic recycling plant into the stream. We investigate differences between organisms living upstream and downstream the recycling plant, differences between species and correlation to sediment concentrations.

3.10.T-07 Where Does the Marine Plastic Go? Abundance and Distribution of MPs in the Kattegat Sea

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Microplastics are ubiquitous pollutants in the ocean and their persistence and potential effects on marine ecosystems is a major global environmental concern. However, we still know little about the distribution and fate of MPs in the marine environment, particularly for the smaller size fraction of MPs ("small MPs"). These "small MPs" (>10 µm) could fatefully overlap in size with the phytoplankton and can possibly be ingested by zooplankton. This study aims to estimate the concentration and distribution of MPs down to 10 µm in the Kattegat Sea. The sampling of fourteen stations in the Kattegat Sea was carried out from the research vessel DANA (DTU Aqua) in October 2020. MP samples were collected in surface waters using plastic-free pump-filter devices ("UFO" sampler). Zooplankton samples (> 200 µm) were collected using multi nets. All the samples were analyzed using µFTIR and the scanned data was processed with the siMPle software. MP concentration in the surface waters ranged between 2 and 77 particles m⁻³. The most abundant polymer particles were Polyester, Polypropylene, and Polyethylene. The concentration of MPs in the zooplankton samples varied from 0 to 3 particles m⁻³, indicating non-bioaccumulation of MPs in the mesozooplankton communities. Our results show a relatively low abundance of MPs in marine waters of the Kattegat Sea and a low ecological risk of physical impacts of MPs on marine planktonic food webs.

3.10.T-08 Extraction and Analysis of 14c-Radiolabelled Polymers From Soil - Results of a Two Years Outdoor Lysimeter Study

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Due to the production volumes of plastics and their application i.a. consumer products, plastics in the environment are becoming increasingly relevant. Worldwide, researchers developed concepts for the determination of microplastics in almost all environmental compartments. In most cases, the focus is on aquatic ecosystems and research on microplastics in soils is still uncommon. One direct application of plastics to agricultural soils is the use of plastic mulch films. In order to fill knowledge gaps, the objective of the presented project "iMulch" was thus to study the fate and the effect of polymers used in agricultural mulch films on organisms and soil ecosystems. Two ¹⁴C-radiolabelled synthesized polymers (polyethylene copolymer (PE) and polybutylenadipat-terephthalat (PBAT)) were tested under realistic conditions in outdoor lysimeters. Soil samples from the lysimeters taken throughout the study as well after cutting the lysimeters in 9 horizontal layers at end of the study were analysed in a first step for their radioactivity content by combustion followed by LSC. For further characterisation different extraction procedures were applied to soil samples depending on the polymer applied. Soil samples from PBAT-lysimeters were subjected to harsh extraction (ASE or soxhlet) with a mixture of CHCl₃ and MeOH in order to dissolve the polymer. PE-containing samples were subjected to both a density separation procedure as well as dissolution at high temperatures with TCB. Quantification of the total extracted radioactivity was performed after filtration of aliquots followed by combustion of the filters. The obtained extracts were analysed by GPC coupled to radiodetection, either at ambient temperature (PBAT) and high temperature GPC (PE). The

recoveries of radioactivity in soil samples taken from the top 5 cm of the lysimeter surface of both the PE and PBAT-lysimeters decreased over the whole incubation period. Analysis of the different soil layers at end of the study showed some translocation of radioactivity. However, nearly no radioactivity was found below 30 cm. Extraction of soil samples from the PBAT-lysimeter with $\text{CHCl}_3/\text{MeOH}$ resulted in good recoveries in the range of 75-90% of the applied radioactivity. The separation of radioactive material from the soil matrix applying a density separation protocol was successfully performed for soil samples containing PE. The extraction of soil samples with TCB is part of ongoing work results will be available on short notice.

3.10.T-09 Microplastic Fiber Emissions From Wastewater Effluents: Abundance, Transport Behaviour and Exposure Risk for Biota in an Arctic Fjord

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Microfibers have been identified as one of the major classes of microplastic found in the environment on a global scale. Very little is known about how they move and distribute from point sources as wastewater effluents into the ocean. We chose the settlement of Longyearbyen on Svalbard as a case study to investigate how microfibers emitted by untreated wastewater will get distributed in the fjord both on a spatial and temporal scale in the remote Arctic. We sampled waste water during two sampling campaigns covering each time the period of one week. We combined the acquired field data with several modelling approaches to include plastic particles into their frame, developed a versatile tool to forecast the fate of the fiber plume leaving the effluent in comparison to abundance of local biomass and their probability to encounter microplastic. The results of this study will add to the knowledge base for future evaluation of a variety of point sources in the Arctic as well as the impact of increasing urbanization and tourism. We used several modelling approaches (FVCOM and OpenDrift) and parameterized plastic fibers into their frameworks, to develop a versatile tool that simulates and forecasts the fate of i) a single fiber plume leaving the pipe and b) continuous emissions from the pipe over one week. We used different densities representing a variety of synthetic fiber materials in our modelling of discharge distribution. The results show that the northern part of the fjord will receive most fibers within 5 days of discharge. Neutrally buoyant fibers will be distributed throughout the water column and also deposit on the seafloor due to hydrodynamic water movement. The tidal cycle will return part of the fibers back into the fjord from the fjord mouth. The implications for pelagic and benthic marine habitats in the fjord are discussed. This pilot study is instrumental in developing tools suitable for future evaluation of a variety of microplastic point sources, as well as the influence of increasing urbanization and tourism on the microplastic distribution in the Arctic.

3.10.T-10 Occurrence and Characteristics of Microplastics in a Small Community Wastewater Stabilization Pond and a University Oxidation Ditch Wastewater Treatment Plant

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Given the high loads of microplastics (MPs) in wastewater, effluent from wastewater treatment plants (WWTPs) can increase the MP burden to recipient waters, even when MP removal efficiencies are >95%. Waste sludge, abundant in microfibers, can also lead to the release of MPs to the environment. Whereas studies investigating MPs in WWTPs have increased, few have examined wastewater stabilization ponds (WSPs), which serve rural and small communities worldwide, and WWTPs that serve universities where sudden and drastic population changes occur. Here, we assessed the distribution and characteristics of MPs in a WSP serving ~500 houses and in a modern oxidation ditch WWTP at the University of Mississippi. Triplicate water samples were collected by rapidly transferring water to fill a 50 L polypropylene container and subsequently passing it through 1 mm, 125 μm and 45 μm sieves. Particles on each sieve were rinsed into 1L glass Mason jars and subjected to Fenton's reagent, followed by density separation, and final filtration. MPs were quantified by stereomicroscopy with a subset of samples identified by focal plane array μFTIR imaging followed by automatic analysis via siMPle software. For the WSP, the predominant polymers detected were polyester and polyethylene. Duckweed and sludge had the highest concentration of MPs, and likely harbor the bulk of the MPs in the system. The concentration of MPs in the treated effluent was lower than in the pond water. There was no seasonal difference in MP abundances. Potential sources of the MPs in the WSP include laundering of clothes, plastics pieces from consumer products washed down the drain, storm water runoff, shedding of paint, and atmospheric fallout. We estimate that the WSP discharges ~786,000 MPs per day in the treated effluent. We conclude that WSPs should not be overlooked as a source of MPs to the environment. For the university WWTP, >90% of the MPs were removed through settling and surface skimming in the primary treatment. Concentrations of MPs in secondary clarifier and final effluent were lower during the higher-flow periods than lower flow periods, likely due to a higher return percentage of sludge to the oxidation ditch and longer process times. MPs were mainly composed of polyester, acrylic paint, polyvinyl chloride, polyethylene, polypropylene and polyurethane. We show that flow rates can have a profound influence on MPs concentrations in the effluent and MP removal efficiencies.

3.10 Microplastics in the environment: Behaviour, transport, fate, risks, and alternatives to conventional plastics (Part III)

3.10.T-11 Investigating Microplastic > 25 μm in the Seine River

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The extensive use and mismanagement of plastic causes environmental pollution in air, soil, and water and threatens ecosystem health and functioning. A large part of plastic in the environment is microplastic (MP), small particles >5 mm. To advance the understanding of MP pollution in river and anthropogenic environments and to provide new insights into the fate of small (25-300 μm) and large (300-5'000 μm) MPs, we investigate MPs in Greater Paris and the Seine river catchment in France. To compare MP concentrations upstream and downstream of Paris we carried out two of four samplings at seven sites along the Seine river in

different flow seasons using a novel in-situ pump and cascade filtration system to increase representativity by sampling larger water volumes up to one m³. In addition we monitored a flood event at an urban site of the Seine river in 2021 over the course of four weeks during the increase, peak, and decrease of the flood. Preliminary data analysis of samples collected upstream and downstream of Paris indicate increased MP concentrations and higher polymer type variation downstream compared to upstream. Among others we detected PP (polypropylene), PE (polyethylene), PS (polystyrene), PVC (polyvinyl chloride), Polyester (including PET), SBR (styrene butadiene rubber), PA (polyamide), PU (polyurethane), Acrylic and ABS (acrylonitrile butadiene styrene). These findings confirm the hypotheses that urban agglomerations contribute to MP concentration in surface water. This trend needs to be confirmed by final data analysis including all samples and size fractions. Final results are foreseen to be complete in early 2022. These studies can help to identify sources and pathways of microplastics in rivers in urban agglomerations and help policy and decision makers to address environmental pollution and mitigation actions.

3.10.T-12 Microplastic Fate in Marine Sedimentary Records of the Ebro Prodelta (NW Mediterranean)

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The accumulation of microplastics in the sedimentary record has developed into the definition of “Plasticene” as a new geological era starting in the 1950s. The current knowledge on the occurrence of microplastics in the ocean points to marine sediments as a major reservoir of microplastic pollutants. However, still little is known on their pathways, fluxes, and accumulation trends in deeper- environments. In the present study, we analyzed one high-resolution sediment core retrieved offshore of the Ebro Delta, NW Mediterranean Sea, for historical microplastic records. To establish the chronology of the last hundred years, radiometric analyses (based on ²¹⁰Pb, and ¹³⁷Cs) were applied. To quantify microplastics and polymer characterization, samples were treated to FPA- μ FTIR-Imaging spectroscopy with automatized microplastic recognition analysis, targeting particles down to 10 μ m in size. Preliminary results show microplastic contamination in sediments spanning the last 70 years, with an increase in the accumulation rate and polymer diversity over time. New results based on the determination of the carbonyl index for the polyolefins, polyethylene and polypropylene, aim to provide new insights into the degradation status of these pollutants once buried in the sediments.

3.10.T-13 Commercial Plastic Degradation Under Seawater Conditions

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Since the use of plastic materials entails certain risks, due to their wide presence in natural environments, new alternatives (bioplastics) are emerging claiming to be more environmentally friendly. However, their behaviour, degradation and potential risks are scarcely described, especially in marine environments. For this purpose, there is a need of standard methods for biodegradability and toxicity assessment under marine conditions. In this study, we tested plastic mineralization in closed bottles and mechanical degradation (weathering) in both small aquariums and large tanks (mesocosms). In the latter, we included pelagic, benthic and littoral exposures. We also assessed toxicity along weathering in *Paracentrotus lividus* larvae, a sensitive marine model organism. Furthermore, two key endpoints for mechanical degradation were measured: weight loss and deformation at break point. The latter is especially useful and it was clearly reduced over time for the compostable bags, while it did not vary for the PE bag. Mineralization, however, was low. Regarding toxicity, it was found that the compostable bags were more toxic than the PE one (2.65 and 2.37 toxic units vs. < 1) and this toxicity disappeared over exposure time, except for littoral treatment, where UV radiation plays a key role. This work helps further understanding biopolymer-based products behaviour in the ocean, contributing to the development of new assessment methods for marine environments.

3.10.T-16 Additives, Plasticizers, and Small Microplastics (< 100 Mm) in Seawater: A Comparison of Three Sampling Methods

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Microplastics (MPs) are ubiquitous, persistent emerging pollutants that have been observed in every environment and in different animal species (from invertebrates to mammals). MPs are part of the micro-litter, together with additives, plasticizers, natural fibers, non-plastic synthetic fibers such as rayon (APFs). The main concern is that invertebrates can ingest all these particles according to the size of their mouthparts, and filter-feeders can collect these particles together with the seston. In particular, all the particles smaller than 100 μ m are actively ingested or filtered; hence, these particles can enter the trophic web, where they can be bioaccumulated and biomagnified. Besides, due to their size, these particles can be transferred from the gastrointestinal tract to other organs or other tissues, such as muscle tissue. Therefore, they can pose a health threat to humans who use them as a food source. There are currently no standardized and harmonized methods for the sampling, pretreatment, contamination control, and analysis of SMPs and APFs (< 100 μ m), which are often overlooked in marine environments. Consequently, a lack of knowledge regards the abundance and chemical characterization of these particles in the seawater and their pathways and fate. Awareness-raising projects and citizen science help spread scientific knowledge and raise the consciousness of more people about crucial environmental issues such as microplastic pollution. SEA Plastics [7] has organized a cruise expedition in the Tyrrhenian Sea and has offered to collect samples to investigate SMPs and APFs. Seawater samples were collected at two sites in South Italy during the 2021 expedition. Three different sampling systems were employed: manta net, plankton net, and Niskin bottles. Samples were

filtered on-site and stored at 4 °C till the arrival at the laboratory of CNR-ISP. An elutriation method was developed to retrieve all the particles collected. A pretreatment method previously developed was performed. All the operations were performed in a plastic-free Clean Room Iso 7. SMPs and APFs were quantified and simultaneously identified via Micro-FTIR. Pyrolysis-gas chromatography was employed as an inter-calibration technique to confirm the identification of specific polymers. The first results show differences among the samples collected with different sampling devices.

3.10.T-17 Effects of Microplastics on Aquatic Community in Freshwater Microcosm

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Microplastics are emerging as a profound scientific and public concern given their abilities to serve as a substrate for growth of microorganisms, to sorb organic pollutants which easily leach into organisms after ingestion, and to travel through and between ecosystems proliferating the entire planet. Research has indicated that microplastics affect living organisms including zooplankton, fish, and several others but has not yet determined their effect on freshwater phytoplankton. This study aims to fill this knowledge gap since phytoplankton are the foundation of the food web in many aquatic ecosystems and invaluable primary producers. Specifically, this study investigates the influence of algal colonization atop the surfaces of microplastics in freshwater microcosms on the following: microplastic deposition rate, phytoplankton community structure, primary productivity, and nutrient availability. Sediment and water were collected from a local pond and added to 9 90-gallon aquaria at Loyola University Chicago to initiate the microcosm systems. After five months of growth and stabilization, microplastics were added in low concentration—for an environmentally relevant comparison—and in high concentration to determine the effects of plastic particles on fully established phytoplankton communities. The addition of plastics initiated the treatment phase. Three aquaria with no addition of microplastics were used as control microcosms for comparison. Additionally, three abiotic aquaria were set up with filtered water to serve as a comparison for microplastic deposition rate with no influence of phytoplankton. Over the course of the treatment phase, monitoring of microplastic deposition, phytoplankton colonization on microplastics, primary productivity, chlorophyll a absorbance, nutrient concentrations, phytoplankton community structure and density, as well as water quality characteristics were conducted. Repeated-measures ANOVA for change in criteria over time and treatments will be used to analyze the significance of any change in the analyzed criteria that takes place after microplastics are added. Non-metric multidimensional scaling will be used to examine changes in phytoplankton communities. A preliminary study revealed that microplastics cause an overall increase in phytoplankton density. Detailed results will be presented at the conference in May.

3.10.T-18 Root System Response of Three Agricultural Crops to Microplastic Type and Concentration

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Microplastics are introduced to agricultural fields through several pathways, including the application of biosolids as a nutrient source. Crops grown in soils with biosolids alter their rooting strategy to uptake the additional nutrients, and alter a number of important traits related to nutrient uptake at the root-system and root segment level, such as the root length density. The attraction of roots to biosolids may increase root exposure to microplastics, especially as smaller microplastics are liable to travel into the rhizosphere and potentially accumulate against the external surface through root water transport and uptake. Surface accumulation may interfere with the uptake of necessary resources from soil, or hinder the excretion of extracellular solutes that are a key element of root foraging strategies and trophic interactions with soil organisms. However, there are a paucity of studies that have investigated how roots respond to soil microplastics and whether root development, architecture, and lifespan are influenced by certain microplastic types and concentrations. Whether roots sense and avoid microplastic alterations to soil properties (e.g., excretion of toxins) remains a considerable knowledge gap. Through a dose-response study, the treatment effects of biosolid microplastics on the root systems of wheat, soybeans, alfalfa were investigated in soil spiked with two plastics at two concentrations 2,000 particles/kg per soil dry weight which reflects ~4 biosolid applications and 15,000 particles/kg per soil dry weight which represents biosolid concentration levels. A control and treatment of direct biosolid application were also included for a total of six treatments. Using the rhizobox approach, plants were grown from seed in a controlled greenhouse. To assess root stress responses, rhizoboxes were scanned weekly over 10 weeks to trace the development of individual roots. Unlike previous studies that assessed microplastic impact at the root system scale the focus of this investigation is higher order roots, as these fine roots are most sensitive to soil properties and their traits align with water, carbon and nutrient cycling processes. Baseline soil microplastic analysis was conducted pre and post planting to determine whether microplastic concentrations were altered by the growing root system. Microplastics in rhizosphere soil were characterized and compared to control soil in terms of the size, shape, type and number of microplastics.

3.10.T-19 Water-Soluble Polymers As New Possible Emerging Concern: Toxicity Evaluation of Polyvinyl Alcohol

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Water-soluble polymers (WSPs) represent a new environmental concern, due to their huge use in many industrial products as detergents, pharmaceuticals, single-use and personal care products. However, no information about the impact of these substances on ecosystems are available. For this reason, the aim of the present study was the evaluation of toxicity induced by a common WSP: the polyvinyl alcohol (PVA). We selected two PVA materials, represented by a PVA powder standard and PVA bag, used as bait container for fishing. Firstly, we characterized the hydrolysis degree as well as the eventual presence of additives in the PVA bag through the Fourier Transform Infrared Spectroscopy (FT-IR) and Nuclear Magnetic Resonance (NMR), respectively. Then, we assessed the chronic toxicity of different concentrations of these materials, solubilized in water, on two different

biological models, represented by the crustacean *D. magna* (exposure of 14 days in semi-static conditions) and the embryos of the teleost *Danio rerio* (exposure from 0 to 120 hours post fertilization - hpf). We evaluated the effects at a high level of biological organization (organism) through the monitoring of the behavioral alterations of exposed specimens, as well as the assessment of the neuro-enzymes monoamine oxidase (MAO) activity. From the chemical point of view, we observed a hydrolysis degree of 85% in the PVA bag, as well as the presence of some additives. Regarding the ecotoxicological evaluation, the exposed specimens of the two species did not show any significant behavioral effects. In the same manner, despite the increasing in the biological trend of MAO, no significant alterations were obtained. However, considering the wide plethora of WSPs, other investigations are necessary to clarify and characterize their ecotoxicological impact.

3.10 Microplastics in the environment: Behaviour, transport, fate, risks, and alternatives to conventional plastics (Virtual Only)

3.1.V-01 Accumulation of Small Microplastics (<100 Mm) and Additives in Gills and Gastrointestinal Tracts of Freshwater Bivalves *Anodonta cygnea*

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The increasing production of plastics and the limitless applications, combined with poor management of waste, generated the widespread global presence of this material in all ecosystems. In particular, freshwaters are very threatened and impacted by microplastics, despite being less studied than marine environments. To fill this knowledge gap, molluscs could be employed to investigate the plastic pollution being filter feeders. Molluscs can uptake microplastics via filtration of seston by gills; these particles (generally < 100 µm, small microplastics, SMPs) can be transferred to the gastrointestinal tract (GIT) and be accumulated in their body. To study accumulation of SMPs, five specimens of the freshwater bivalve, *Anodonta cygnea* Linnaeus, 1758 were bought; then they were exposed in cages in three rivers located in the Lazio Region (Italy), River Marta, River Aniene, River Sacco, to different environmental conditions for short range (one month) and for long range (three months) exposure. After the environmental exposure (i.e., short range and long range exposure), specimens were dissected in laboratory. The gills and the GITs were removed and analyzed separately to evaluate the possible uptake and ingestion of SMPs. The pre-treatment of gills and GITs was performed in a plastic-free clean room ISO 7 (CNR-ISP). All the steps of the pre-treatment procedure allowed to not further denature SMPs and additives, so as to avoid over- and under-estimation of the particles and for an optimal polymeric identification. After chemical digestion, the slurred samples were filtered on alumin oxide filter membranes (0.2 µm, 47 mm diameter) and the analysis for the quantification and simultaneous polymer identification were performed via Micro-FTIR. The polymers most found were nylon, widely used in clothing, fishing and agriculture fabrics, and olefin, used in carpeting, ropes, and vehicle interiors due to its resistance to staining, mildew, abrasion, and sunlight. Several plastic additives such as Varox DCP-40C, a vulcanizing additive, N-(2-ethoxyphenyl)-N(2-ethylphenyl)-ethanediamide, a stabilizing plasticizer, and poly(N-methyl acrylamide), a thermoresponsive additive, were found in the samples. The preliminary results highlight an environmental pollution by SMPs using freshwater bivalves as sentinel organisms.

3.1.V-02 Assessing the Role of Polyethylene Microplastics As a Vector for Organic Pollutants in Soil: Ecotoxicological and Molecular Approaches

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Microplastics (MPs), pharmaceuticals and pesticides are emerging pollutants with proposed negative impacts on the environment. Rising interest in investigations of MPs is likely related to their potential to accumulate in agricultural systems as the base of the food chain. In this study, the ecological stress of MP contamination, alone or associated with other environmental pollutants, on soil was evaluated. We applied an integrated approach using classic bioassays and molecular methods to evaluate the impact associated with a mixture of three types of polyethylene (PE) microbeads, and their interactions with organic pollutants (OCs), including ibuprofen (IB), sertraline (STR), amoxicillin (AMX) and simazine (SZ), on different soil organisms. PE-MPs exhibited different abilities for the adsorption of each tested OC. Standard soil was artificially contaminated with OCs and MPs (alone or combined with OCs) and incubated for 30 days. The presence in soil of MPs, or MPs and OCs (MIX), did not produce any effect on *Caenorhabditis elegans* endpoint growth, reproduction, or survival. Inhibition of leaf growth in *Zea mays* was detected, but this negative effect declined over time, while the inhibition of root growth increased, especially when OCs (32 %) or MIX (47 %) were added. Moreover, the expression of the antioxidant genes *CAT 1*, *SOD-1A* and *GST 1* on plants was affected by the treatments studied. The addition of MPs or MIX affected the soil bacterial phylogenetic profile, which selectively enriched specific members of the bacterial community (particularly *Proteobacteria*). The predicted functional profiles of MP/MIX samples indicated a potential impact on the carbon and nitrogen cycle within the soil environment. Our results indicate that MPs and their capability to act as pollutant carriers affect soil biota. The synergistic effects of both types of elements are particularly relevant for plants. Considering that the maintenance of healthy ecosystems relies largely on the key role of plant and microbe interactions, particular attention may be paid to agricultural systems as the base of the food chain. Therefore, further studies should be carried out with different types of MPs to establish their respective interactions with organic compounds, their bioavailability and how long it takes for leaching of these organic pollutants into different organisms and/or ecosystems.

3.1.V-03 Assessment of Eleven Phthalate Acid Esters in Mediterranean Edible Fish Species in Relation to Microplastic Ingestion

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The relationship between the presence of microplastics and plasticizers level in the environment and marine organisms is a new area of research under development. Phthalate acid esters (PAEs) are chemicals widely used as plasticizers to improve the properties of plastic materials. It has been shown that they can be easily released into the environment and reach all environmental compartments. PAEs are considered a class of pollutants that cause significant damage to the environment, wildlife, and humans. However, few studies have assessed the presence of phthalates in commercially important fish species. This study aimed to evaluate and compare the levels of PAEs in the muscles of edible fishes, from the North-Western Mediterranean Sea, in relation to microplastics ingestion. Microplastics ingestion was evaluated in *Boops boops*, *Engraulis encrasicolus*, *Mullus surmuletus*, *Sardina pilchardus*, collected in areas affected by different anthropic pressure of the North-Western Mediterranean Sea. The abundance of 11 PAEs (Dimethyl phthalate, Diethyl phthalate, Diallyl phthalate, Dipropyl phthalate, Diisobutyl phthalate, Dibutyl phthalate, Benzyl butyl phthalate, Dicyclohexyl phthalate, Bis(2-ethylhexyl) phthalate, Di-n-octyl phthalate, Diisononyl phthalate) were investigated in a sub-sample of each species, properly selected in order to compare contaminants loads among organisms with and without microplastics, for a total of 104 specimens (30 *B. boops*, 30 *E. encrasicolus*, 16 *M. surmuletus*, 28 *S. pilchardus*). Results show that all the PAEs investigated were detected in an appreciable amount in the 4 species. The PAEs compositions were different in each species, the most founded were Diisobutyl phthalate, Dibutyl phthalate, Bis (2-ethylhexyl) phthalate. Specimens of *B. boops* had the highest levels of PAEs. No differences in the abundance and composition of PAEs were observed in relation to microplastics ingestion. However, distinctions were highlighted for specimens belonging to species and from specific areas affected by different microplastics impacts.

3.1.V-04 Assessment of Microplastics in Indus River System, Pakistan

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Indus River apart of being one of the longest rivers of Asia is also the main source of freshwater supply in Pakistan. The following research focused on the quantification and characterization of microplastics in this river system with primary focus on selected glaciated and freshwater lakes including: Batura lake, Borith lake, Naltar lakes, Attabad lake, Khalti lake, Passu lake and Upper kachura lake. The preliminary results reveal the occurrence of plastic particles in all glaciated and freshwater lakes. The presence of fibres in microscopy indicates the impact of tourism and anthropogenic activities in the surrounding, however, the presence of microplastic content could indicate both the anthropogenic and atmospheric origin of contamination. The fibre content in the glaciated lakes was found to range from 2 fibres/Kg to 180 fibres/Kg of dried sediments, whereas for surface water the fibres ranged up to 27 fibre/L of surface water. Although the comparing of the results from different lakes does not suggest a specific trend or reason of occurrence, however, the sites more laden with anthropogenic activities were found to be more fibre laden. From the microplastic analysis (referring to the specificity of studied lakes), the mainly occurring microplastics were polypropylene (PP) and polyethylene (PE) accompanied by polystyrene (PS), Poly vinyl chloride (PVC) and **Polyethylene terephthalate (PET)** at various sites.

3.1.V-05 Cladophora Spp. Could Interact With MPs Particles?

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Macrophytes can trap microplastics (MPs) in surface water ecosystems, mainly working as a net through roots and filament. In a previous study, presented at Seatac 2020, we studied the environmental fate of these new contaminants, finding in macrophytes sample an MPs concentration higher than in other matrices analyzed (water and sediment). The study was performed in two watercourses in the natural area of Farfa river and in a highly impacted site, located in the urban area of Rome, Almone river. In the current study, we investigate, in vitro, the interaction between MPs, pellet, foam and fragment, and a filamentous alga (*Cladophora* spp.) both sampled and isolated in the laboratory from raw environmental samples (water, sediment and plants). *Cladophora* and MPs were placed in a multiwell plate, with filtered river water as a growth medium, at 18-20 °C with a 16h light and 8h dark photoperiod. In each multiwell, two wells were dedicated to negative controls (without MPs), two wells with low concentration and the last two as high concentration of MPs. Comparing the control sample with the treated ones, preliminary results show that *Cladophora* growth is not significantly influenced by the concentration of MPs. Moreover, this alga's remarkable capability to adhere and trap the MPs particles, functioning as traps during an algal bloom. Further analyzes are underway to detect any cellular interactions between this algae and MPs.

3.1.V-06 Comparison of Three Sampling Methods for Microplastic in Rivers

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Despite many studies and different estimates on microplastic occurrences in rivers, microplastic analysis is still a young field of research with many challenges. However, one of the main difficulties lies not least in a reliable representative and thus comparable sampling. Above all, the isolation of microplastic particles from the sample matrix of environmental samples before detection is challenging. In river samples, there are comparatively few microplastic particles in a heterogeneous and complex matrix of organic and inorganic bycatch of different grain sizes, which have to be separated and analysed by means of different

preparation steps. Currently, there is no standard method used to sample microplastics from riverine systems. The presented study therefore focusses on different sampling methods for microplastics in rivers and shows empirical data from sampling in the Danube comparing three different sampling techniques and their possible influence on subsequent sample preparation and evaluation. In the Tid(y)Up project, different microplastic sampling methods were compared for the first time at 8 different sampling sites (in Austria, Hungary, Serbia, Romania and Bulgaria) along the Danube and Tisza. A further developed net method, taking into account the depth variance and spatial distribution over the transverse profile (Liedermann et al. 2018), the so-called cascade pumping method (higher resolution for small particle sizes) and a sedimentation box (temporal component through measurement over 2 weeks) were used. The focus of these measurements was on the practicability of these established measurement techniques in terms of implementation, user-friendliness, susceptibility to errors, personnel requirements, etc. depending on the fluvial framework conditions as well as subsequent following requirements for sample preparation. The methods show different advantages and disadvantages in their application. However, it seems essential that each method involves different sample preparation steps. The challenge in sample preparation lies primarily in isolating the plastic particles from all other unwanted (in)organic residues without altering or even destroying the MP particles in any way. Harmonised protocols or standardised approaches for quality assurance and quality control in the sampling and evaluation of microplastics have to be developed.

3.1.V-07 Following the Fate of Microplastics: Why Are Wastewater Treatment Plants Two-Edged Swords?

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Microplastic (MP) pollution is ubiquitous in the environment presenting a global problem of both scientists and general public. One of the major pathways of microplastics entering the natural environments are through wastewater treatment plants (WWTPs). Once microplastics reach natural environment, they are posing treat to aquatic ecosystems and public health. Still to date, there are no strict regulations regarding its monitoring and waste management. The aim of this study is to investigate concentration, morphology and composition of microplastics in different treatment units of WWTP. Sampling included different points of WWTP in the water line and sludge. Pre-treatment of the samples consist of the advanced Fenton oxidation, alkaline and enzymatic digestion, in order to remove organic matter. A density separation was applied using zinc chloride solution. Once the particles were isolated, their morphology and size were studied using a stereoscopic and optical microscope. Spectroscopic techniques such as ATR-FTIR and micro-FTIR were used for the analysis of the polymer's composition. Results showed that removal efficiency of WWTP is high and exceed 99%. Most abundant morphology is fibers that are coming from synthetic materials during washing processes. The most abundant polymers are: PE, PP, PVC, PE-PP copolymer, polyethylene-ethyl-acrylate copolymer, and for fibers: polyester, polyamide and cellulose. Considering the amount of wastewater treated annually (12,892,235 m³/year) and the average number of microplastics removed per litter for both sampling periods (710 MPs/L), it can be concluded how much microplastics are avoided from being emitted into the environment through direct water discharge (9,147,685 MPs/year). Removed MPs will accumulate in the sludge that is used in agricultural purpose although it should be managed as waste properly, avoiding transition of microplastic pollutants to terrestrial ecosystems adding to the number of MPs that will inevitably end up in receiving water bodies through this route (3,744,549 MPs/year).

3.1.V-08 Long- Term Impacts of Microplastics From Disposable Medical Masks on Terrestrial Invertebrates

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The global coronavirus pandemic crisis has increased the consumption of disposable medical masks, which may represent an important source of microplastics in the environment. The aim of this study was to investigate microplastics from polypropylene medical masks. Three layers of the masks were separately milled and characterised. Each of the inner frontal, middle filtering, and outer layers yielded different types of microplastics: fibres were obtained from the inner and outer layer, but irregular fragments from the middle layer. The particle diameters according to laser diffraction analysis were 45.1 ±21.5 µm, 55.6 ±28.5 µm and 42.0 ±17.8 µm for inner, middle and outer layer, respectively. The chemical composition of plastics-associated chemicals varied between the different layers. The inner layer contained more chemicals related to antimicrobial function and flavouring, the other two layers also contained antioxidants, plasticisers, cross-linking agents, antistatic agents, lubricants, and non-ionic surfactants. We investigated the effects of long-term exposure to these microplastics in soil on the woodlice *Porcellio scaber*, mealworms *Tenebrio molitor* and enchytraeids *Enchytraeus crypticus*. The immune response of woodlice after 7 and 21 days, energy-related traits (electron system activity, lipids, carbohydrates, proteins) of mealworms, and reproduction of enchytraeids after 21 days exposure were evaluated. None of the three microplastic types affected the survival of the invertebrates, but other physiological changes were observed. Woodlice responded by induction of immune response, which was more pronounced after 7 days at the highest concentration tested (1.5%; w/w), but most of the parameters returned to normal levels after 21 days. In general, no significant shifts in energy allocation were observed in mealworms, but some of the parameters were changed sporadically. Lipids were only increased at 0.06% and 0.5% of inner layer particles, protein content was increased at 0.5% of middle layer particles, but electron system activity was increased at 0.5% and 1.5% of outer layer microplastics. Carbohydrates levels were not changed. No effects on the reproduction of enchytraeids were observed. We could not find a clear difference in effects between the microplastics from the three layers despite their different shape and organic chemicals composition. We conclude that organisms respond to the presence of microplastics in soil, but changes are not adverse.

3.1.V-09 Micro- and Macroplastic Concentrations in the WATER Column and Sediment of Belgian Sea Ports and Estuaries

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Society is surrounded by a wide range of plastic objects, ranging from everyday items to complex products and machines. During production, use and waste management, there is a potential emission of plastics of all sizes (micro – macro) to various environmental compartments. This includes emissions to the environmental compartments such as air, soil, freshwater and the aquatic environments. Plastic particles and filaments are known to settle in riverine sediments, and/or to flow downstream and eventually reach the marine environment. Until now, there is however little information about the three-dimensional distribution of plastic in water bodies, including the influence of abiotic conditions in plastic debris fate. Within the PLUXIN project, one of the objectives is to map and quantify plastic waste accumulation zones and the plastic flux from Flemish estuaries and ports to the southern North Sea. More than 130 microplastic (100 µm – 5 mm) and 260 macroplastic samples have been collected in the water column and sediment at 20 sampling locations in the port of Nieuwpoort (Yser estuary), port of Ostend, port of Antwerp, North Sea Port and the Scheldt estuary in Belgium. The sampling strategy included three types of campaigns to account for spatial and temporal variation in the concentrations of plastics in different environmental compartments. We performed: (1) seasonal multi-day campaigns; (2) 13h tidal cycle measurements; and (3) bimonthly spot sampling campaigns. We report on in situ observations of micro- and macroplastic, including their horizontal and vertical distribution in the water column and their presence in the sediment at predefined locations. In addition to location-specific concentrations, the polymer types and size-frequency distributions of the simultaneously sampled micro- and macroplastics are determined.

3.1.V-10 Microplastic Contamination in Flanders: Identification of Sources, Pathways and Mitigation Strategies

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Plastic industry is a booming business but unfortunately, part of the produced plastics end up in the environment. Once in the environment, the plastic will fragment into small particles, called microplastics (MP, smaller than 5 mm). Despite large (inter)national research efforts, many questions remain unanswered, especially at a local or regional scale. Therefore, the aim of the current study was to explore the MP pollution in the freshwater environment in Flanders. This research processed and analysed 210 samples collected from eight different matrices distributed in the geographic region Flanders (Belgium). Plastics were identified using Fourier-transform infrared spectroscopy. It was evident that MP are omnipresent in the freshwater ecosystems of Flanders. One litre of surface water contained 0.48 MP particles (ranging between 0 and 4.8 MP per litre). The sediment of the waterways contained on average 2,990 MP particles per kg of dry weight sediment (ranging between 610 and 9,558 MP per kg). The risk of adverse effects of MP pollution for the Flemish waterways are low to negligible. The current research quantified the MP present in domestic waste water as one of the sources of MP pollution. Per litre of domestic waste water, 0.96 to 39.8 microplastic particles were found. In 83% of the households, the domestic waste water is being transported to an active waste water treatment plant (WWTP), which are able to remove 97.5% of the MP before discharged in the waterway. Another source of MP contamination in the environment are the microscopic rubber tire wear particles that are formed due to the friction between the tires and the road. Based on the run-off samples, an estimated emission of 10.8 mg tire wear particles per driven km was calculated. The emissions of both above mentioned sources were extrapolated to whole Flanders region area. From the yearly MP pollution in the domestic waste water, 623 kg of MP particles will end up in the aquatic environment. The highest losses originate from the households that are not connected to a WWTP. The yearly net emission of tire wear particles in Flanders is estimated to be 245,926 kg, a remarkably higher emission compared to the estimated MP emission from domestic waste water. In conclusion, this research was able to demonstrate that MP are ubiquitous in the Flemish environment, but this data offers some clear perspectives on mitigation measures to reduce MP emission to the freshwater ecosystem.

3.1.V-11 Microplastic Distribution in Seagrass Sediments From a Tropical Atoll, Belize

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Microplastics (MPs) are widespread, but the key factors affecting their fate and distribution remain to be fully elucidated. One of the factors that is believed to significantly influence microplastic transport and sequestration is the presence of aquatic plants, including seagrasses, which reduce the velocity of water flows leading to sediment deposition. For this study, we collected sediment samples from seagrass meadows on the Turneffe Atoll, Belize, with replicate samples taken from four sites representing a range of anthropogenic and hydrodynamic influences. Each sample was collected within a quadrat, in which % seagrass cover was estimated using drone imagery. It was hypothesised that: a) microplastic concentrations would vary between sites due to broadly different anthropogenic influences and hydrodynamics (affecting site energetics), with high energy sites containing fewer MPs due to less deposition of particles overall, b) increased seagrass cover would lead to higher concentrations of MPs in the underlying sediments, and c) smaller-grained sediments (and thus lower energy environments) would be associated with higher local concentrations of MPs. Samples were processed using an oil separation method to separate MPs from inorganic particles, followed by H₂O₂ digestion to remove organic material. Samples were analysed using 25 µm resolution µFTIR and spectral maps processed using siMPle software. Sediment grain size was measured for each sample. Contrary to hypothesised, microplastic

concentrations did not vary significantly between sites, nor did seagrass cover influence concentrations. Concentrations within samples were highly variable, even within sites, suggesting that the conditions affecting microplastic accumulation can be highly localised, and highlighting the importance of replication within sites for MPs studies in this location, and in general. Sediment grain size was significantly correlated with microplastic concentration but in the opposite way to expected, with a higher proportion of larger grained sediment related to a higher concentration of MPs. This suggests that MPs are deposited more readily in higher hydrodynamically energetic environments compared to lower energy environments and thus cannot be considered as sediment analogues. This unexpected behaviour may be related to variable particle density as a result of polymer type, or wider biological interactions such as biofouling and aggregation.

3.1.V-12 Microplastic Pollution in Drinking WATER in Flanders

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Recent studies described the occurrence of microplastics in nearly all environmental aquatic matrices. As expected, microplastics have also entered the food chain and can be ingested by humans via food and beverages such as salt, beer, drinking water and fruits. The purpose of this study was the identification of microplastics in large volumes of tap water that were derived from the purification of ground water, surface water or purified wastewater effluents in a densely populated region in western Europe, i.e. Flanders (Belgium). The samples were collected across Flanders at two different places in the supply chain: (1) purified water from drinking water treatment plants and (2) drinking water from conventional household taps. To identify potential microplastics, Fourier transform infrared microscopy was performed which enabled the identification of microplastic particles down to a size of 25 µm. A rescaling of the concentrations, based on a published method, was performed to enable the calculation of the microplastic concentration in the default microplastic range (1µm-5000µm). An average of 0.02 ± 0.03 microplastics (>25µm) per liter (ranging between 0 and 0.06 microplastics per liter) was found in the samples taken in the water production centers. In three different water treatment plants – Essen, Egenhove and Gavers – no microplastic particles were found. Polypropylene is most commonly found polymer type after purification of the source water. Relating microplastic concentrations to the origin of the water (surface water, ground water and purified waste water), we observed that drinking water produced from groundwater did not contain microplastics in our samples. The drinking water from waste water effluents contains on average more microplastics (0.05 ± 0.02 MP/L) than that from surface water (0.02 ± 0.02 MP/L). However there is no significant difference in microplastic contamination according to the source of the water (p = 0.08). More data should be collected to confirm these results. It is not (yet) possible to assess potential risks of microplastics ingestion for human health as no epidemiological or other relevant studies on the effects of ingested microplastics have been published. More research is necessary to calculate a Derived No Effect Level (DNEL) for risk assessment of microplastic intake for human health.

3.1.V-13 Polyethylene Microplastics Induce Neurotoxicity in Terrestrial Snail but There Are No Effect on Behavior

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Microplastic pollution has been rising in soil ecosystem. This study confirmed the effect of ingesting polyethylene microplastics in giant African snail (*Achatina fulica*). We fed microplastics with food to snails every two days for seven weeks. We observed that how much time spent snails looking for food. After exposure we sampled cerebral ganglion and measured acetylcholinesterase activity (AChE activity). We found that ingest of microplastic could decrease the AChE activity of snail which is the biomarker of neurotoxicity. But compared with previous study, behavioral disorder did not appear. This study found that continuous intake of microplastic cause neurotoxicity on snail in terrestrial ecosystem. *Acknowledgement- This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning(2020R1A2B5B02001734).*

3.1.V-14 Surfactant Agents in Micro- and Nanoplastic Research: Friend or Foe?

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As microplastics are posing a challenging problem for the environment, there is an increasing need for relevant studies (both in terms of exposure and effects) on the effects on humans and (aquatic) animals. Within this context, the availability of standard reference micro- and nanoplastic particles is recognized. Small micro- and nanoplastics in various shapes and size can be produced by cryomilling, however, a main challenge appears to be linked to the hydrophobicity of the plastics resulting in insoluble plastic particles. Surfactant agents such as Triton X-100 are often used to reduce hydrophobicity and thus increase solubility of the plastics. Nonetheless, these surfactants could in their turn affect cells or organisms during experimental exposure causing unreliable results. The goal of this study was to identify and recommend surfactant agents to be used to prepare test solutions of micro- and nanoplastics reference materials for exposure studies. The performance of the surfactants was studied by analysing the capability of reducing the hydrophobicity and the toxicity of the surfactants. The latter was tested on human colorectal adenocarcinoma (Caco-2) cells and alveolar cells (BEAS-2B), the two most important intake pathways of micro- and nanoplastics. To understand the possible toxic effects of surfactants on the (physiology of the) cells, four different assays were used to determine the mitochondrial activity, total protein count, cell viability, and reactive oxygen species (ROS)-production. Tests were initiated by seeding cells at 20,000 cells/well in a multiwell plate and grown for 24 hours. Subsequently, the cells were exposed to a concentration range of surfactant ranging from 1 x 10⁻⁶ mg/ml to a maximum 5 mg/ml. After a 48 hour exposure period, the four previously mentioned assays were performed. The results clearly indicate surfactant-specific effects on the cells, and Triton X-100 shows the highest cytotoxicity.

3.1.V-15 Whereabouts of Microplastics and Their Flux Towards the Marine Environment

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Plastics are globally dispersed and reported at increasing concentrations in marine ecosystems. Due to their persistence in aquatic environments the global plastic litter problem will last for decennia. Hence, plastic detection methods and plastic remediation measures are urgently needed and may become obligatory in the near future. A first prerequisite to take effective plastic remediation measures is to know where and when action should be taken. However, to date there is a critical knowledge gap about the whereabouts of plastics and their flux towards the marine environment. Part of the PLUXIN project aims to close this knowledge gap by using numerical modelling to gain insight into the fate and transport of plastic debris across environmental compartments (www.pluxin.be). The model is calibrated and validated using field sampling and experimental data. More than 130 microplastic (100 μ m – 5 mm) samples have been collected at 20 sampling locations in port of Nieuwpoort (River Yser), port of Ostend, port of Antwerp, North Sea Ports and the river Scheldt in Belgium. Information on microplastic concentrations in the field was combined with settling experiments to study the behavior of microplastics in the water column by determining their vertical flux as a function of polymer type, shape, size, degree of biofouling, and weathering. A depth-averaged, two-dimensional-horizontal coupled Eulerian particle transport model for the Scheldt Estuary and Belgian Coast was set up using open-source TELEMAC software. Based on the preliminary results accumulation hotspots were pinpointed and the total flux from rivers and harbors towards the marine environment can be quantified.

3.1.V-16 Microplastics and the Water Industry: Studying Source, Transfer and Fate Within the Microplastic Cycle

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Biosolids are recovered as a by-product from sewage treatment works and, in the UK, are typically used as a fertilizer and means of returning organic matter to agricultural land. Application of biosolids is potentially a major source of microplastics in soils as the biosolids can retain over 90% of the microplastics present in the wastewater entering a sewage works. This study will assess the presence of microplastics in sewage sludge produced by sewage treatment plants in the south west of England. Microplastics will be extracted from the sludge by density separation using saturated ZnCl₂ followed by peroxide digestion of organic matter and filtration. Microplastics will be retained on the filter paper. The size, shape and colour of the microplastics will be determined using stereomicroscope observations; Fourier Transform Infrared Spectroscopy will be used to identify the polymer types present. The same approach will be used for soil samples obtained from agricultural soils where biosolids have been applied. Soil from agricultural land that has not had biosolid applications, but which has similar land use history and similar chemical, physical and landscape scale properties will be used as controls for identifying non-sludge derived microplastics. Site selection will be based on data gathered from farmers and data records held by the sewage treatment plant. This project will also investigate the potential risks of microplastics to soil inhabiting invertebrates, alteration of soil properties due to the accumulation of microplastics and whether microplastics are likely to be retained in the soil or transferred into aquatic systems via runoff from agricultural land contributing to diffuse pollution downstream.

3.10 Microplastics in the environment: Behaviour, transport, fate, risks, and alternatives to conventional plastics (Poster)

3.10.P-We061 The Occurrence of Waterborne Pathogens Colonizing Different MPs Polymers

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Microplastics (< 5 mm, henceforth designated as MPs) have been shown to serve as a new habitat for microbial community, by forming the so-called “plastisphere”. The colonization of MP surface by biofilm-forming bacteria is a coin of double face since this biofilm can contain human and animal pathogens (e.g., the genus *Vibrio*) and hydrocarbon-degrading bacteria, which can potentially influence the fragmentation and degradation of plastic waste. It has been shown that this community differs from the community in the surrounding waters and plastic polymer types. Thus, this study aims to disclose the microbial communities forming the plastisphere in different types of microplastics. To accomplish this, the microbial community was isolated from two impacted aquatic systems and incubated with 1-2 mm MP particles of 5 different plastic polymers (polypropylene (PP), polystyrene (PS), expanded polystyrene (EPS), polyethylene terephthalate (PET) and polyethylene (PE)). After 1, 3, and 7 days of incubation at room temperature with a low-speed shake (100 rpm), MP samples were collected for: (a) DNA extraction and quantification; (b) extracellular polymeric substances detection and quantification; (c) 16S rRNA amplification (PCR and metabarcoding with 16SV1V3 and 16SV3V5 primers). After 30 days of incubation, MPs particles were collected for analysis by FTIR spectrum. The results showed the existence of DNA and extracellular polymeric substances associated with all five MPs particles assessed. The highest amount of DNA quantified was found in the PET particles while the PP category showed higher polymeric substances formation. Concerning the microbial community composition, a diverse community composed of *Vibrio* sp., *Pseudomonas* sp., *Catenococcus*, and *Shigella* sp., was found, with *Vibrio* sp. showing higher occurrence. Some pathogenic species showed high similarity with the amplified sequence, including *V. campbelli*, *V. parahaemolyticus*, *V. fortis*, *V. ichthyenteri*, *V. pelagius*, and *S. dysenteriae*. Higher diversity was found for PS particles. The FTIR spectrums showed some differences

compared with the MPs control. Thus, this study strengthens the impact of MPs particles, including those from different polymers on aquatic systems by acting as a vector for aquatic life exposure to pathogens.

3.10.P-We062 Biodegradation Test Methods to Assess Microplastic Materials: A Multi Compartment Study Using Polyester-Based Materials

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The ECHA restriction proposal on intentionally added microplastic exempts biodegradable microplastic materials from bans, labelling and reporting. To determine the biodegradability of microplastic materials, a tiered approach has been proposed by ECHA based on OECD testing guidelines and ISO standard methods. In these tests different inoculum sources can be used such as sludge from sewage treatment plants, soil, marine water and/or sediment. One of the key questions is if these test methods can be considered equivalent, since a systematic evaluation is still missing. In this study we investigated the biodegradation of a polyester blend applying different test methods that are included in the ECHA microplastic restriction proposal. To validate the environmental relevance of these methods, in parallel, field studies have been performed on macroscopic samples of the same material. In addition, kinetic modelling of the results was performed to better understand relevant parameters affecting biodegradation. Our results show a positive correlation between lab and field studies, demonstrating the environmental relevance of the ISO methods developed in the recent years for the testing of biodegradable materials. Furthermore, the data presented in this work show that the investigated material can be biodegrade in all different environmental compartments to the same extent as the positive control. Finally, the developed model gives valuable insights into the understanding of the biodegradation mechanisms.

3.10.P-We063 A Full Year of Atmospheric Microplastic Monitoring in a Remote High-Altitude Catchment Area in the Pyrenees, Southwestern France

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Atmospheric deposition of microplastics to mountainous areas has been demonstrated by Allen et al., 2019. This study aims at pursuing these investigations, with a focus on seasonality, deposition form (wet versus dry deposition), as well as the altitude effect on atmospheric deposition of microplastics. In addition, Microplastic distribution and quantity will be intercompared with trace element content to help determine whether some trace elements act as proxies for specific synthetic polymer types. From 26/11-2019 to 07/10-2021 rainwater as well as dry atmospheric deposition have been collected on a monthly basis, from an automated rainwater/dry deposition collector. The collector was placed in a natural mountainous area in the Pyrenees in Bernadouze, France at an altitude of 1425 meters. A total of 24 rainwater and 24 dry deposition samples were prepared for automated Raman microscope analysis. Samples synchronously collected in the state of Andorra, at similar conditions, will later be compared to these findings. From each sample 1000 ml of rainwater was filtrated unto 0.45 µm Millipore Teflon filters, and the content transferred into vials with 30% H₂O₂ for the degradation of biogenic material. After 7 days of digestion at 50°C the content was once again filtrated unto 0.45 Millipore Teflon filters and transferred into a 1.6 g/cm³ ZnCl₂ (Zinc Chloride) solution. Here the heavy fraction i.e. lithic fragments, was gradually sorted out by repeatedly shaking, settling and flushing the contents of a glass decanter. After separation, the light floating fraction was filtrated unto 1 µm, 10x10 mm pure silicon membrane filters and thoroughly rinsed with milli-Q deionized water. The silicon filters were analyzed using the automatic scan function of the Horiba Soleil Raman microscope. The instrument is capable of measuring particles down to 1 µm; a critical feature when analyzing atmospheric particles. Results have yet to be procured but will be ready in May 2022.

3.10.P-We064 Exploring the Potential of Raman Microspectroscopy for the Analysis of Microbial Degradation of Microplastics

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Biodegradation of microplastics in the aquatic environment is not studied sufficiently to predict the fate of microplastics – synthetic polymer particles in the size range 1 µm – 1 mm. Mainly indirect methods like mass loss of the polymer or changed polymer properties have been investigated, which give information about microbial colonization and enzymatic depolymerization. Alternatively, CO₂ formation during microbial utilization of hydrolysis products is commonly monitored. However, the most direct line of evidence - biomass formation out of plastics - has only once been investigated by Zumstein et al.: carbon from synthetic polymers in soils was traced into microbial biomass using stable isotopes and nanoscale secondary ion mass spectrometry (NanoSIMS). In this work, Raman microspectroscopy (RM) will be applied with the aim to directly monitor the uptake of isotope labels from the polymers into the biomass on a single cell level in a non-invasive way: application in water samples can enable to directly detect phenotypical heterogeneity within the microbial community. While NanoSIMS has excellent lateral resolution, RM persuades with easy sample preparation, non-destructiveness, water insensitivity and structural information, which offer to trace isotope labels into different cell compartments. For a first study, the bacteria *Sphingomonas koreensis*, which were isolated from an environmental aquatic sample, were chosen due to their potential to biodegrade Poly(lactic acid) (PLA) particles. *S. koreensis* cells contain carotenoids in various cell compartments, which can be used for resonance Raman due to their chromophoric system. This way, Raman spectra can be obtained in only 1 s with a laser power of 1 mW at the sample. Quantitative ¹³C labeling of carotenoids was previously shown by the group of Huang. Since isotopically labeled polymers are very expensive or not commercially available, a reverse labeling approach will also be tested by initially labeling cells with ¹³C-

glucose. The resulting red-shift can be linearly assigned to the according ^{13}C -content of the substrate. For the first experiments PLA, the most common biodegradable plastic, is chosen as single carbon source. While this procedure will be used as cheap method to indicate whether single bacteria cells are able to metabolize a specific polymer by shifting the label bands back to the initial wavenumber, a direct labeling approach will be required for environmental samples. Therefore, D- and ^{13}C -labeled polymers will be used in a following direct labeling approach.

3.10.P-We065 Determination of Microplastics in Moss Using Thermal Extraction- Desorption Gas Chromatography Mass Spectrometry Ted-Gc-Ms

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The use of mosses as biomonitor for several atmospheric pollutants like heavy metals and persistent organic pollutants is well established in the literature. To investigate whether moss can also be used as biomonitor for atmospheric microplastics deposition, a method for quantifying microplastics in moss using thermal extraction - desorption gas chromatography mass spectrometry (TED-GC-MS) was developed. Within the sample preparation a homogenization of the young plant parts and an oxidative digestion using the Fenton reaction to reduce the matrix followed by a filtration step was performed. For validation six different polymers, polyethylene (PE), polystyrene (PS), polyamide (PA), polypropylene (PP), Polyethylene-terephthalate (PET), Styrene-butadiene-rubber (SBR) were added to 1 g (dw) moss samples (*Pseudoscleropodium purum*) and recovery rates were determined. The calculated recovery rates ranged between 84 and 93% with a standard deviation between 4 and 9%. After validation, environmental moss samples from different sampling sites in Germany (Lower Saxony, North Rhine-Westphalia, Rhineland-Palatinate, Baden-Württemberg) were analyzed. Microplastics could be identified and quantified in all environmental moss samples. This method will be applied in a pilot study as part of the German Moss Survey 2020/2021 supported by the German Environment Agency (UBA, Research code 3720632010) to quantify microplastics in environmental moss samples from further 20 sampling sites.

3.10.P-We066 Microplastics in Total Atmospheric Fallout: Monitoring in Peri-Urban and Agricultural Sites

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Microplastic (MP) pollution has been the source of an increasing scientific interest since the beginning of the century. While MPs have been described in virtually environment, some environments remain less studied and less understood than others. In particular, the occurrence of microplastics in the atmospheric compartment has only been studied for a few years. This work presents the result of three total atmospheric fallout monitoring campaigns conducted in two different sites in the Paris region. Total atmospheric fallout was collected using passive sampler for 4 to 6 month for the different studies. Samples underwent a density separation followed by a chemical treatment, before they were analysed using an automated μFTIR mapping analysis using a Nicolet iN10 by Thermo Scientific. MPs could be identified down to a size of 25 μm , cutoff point determined by the μFTIR detectors. Preliminary showed deposition rates of a few dozen MP/m²/d. In the case of two fully analysed samples from site A in 2021, deposition rates reached 9.5 and 48.9 MP/m²/d. Other samples currently await μFTIR analysis. These results were compared to earlier studies conducted in the Greater Paris region.

3.10.P-We067 Caddisfly Larvae As a Novel Biological Source of Microplastics in Freshwater Systems

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Plastic pollution is ubiquitous in freshwater environments globally, with many invertebrates known to interact with both micro- and macroplastics. Caddisfly larvae (order Trichoptera) utilize material from their environment to build portable cases, and previous studies have found that some species incorporate microplastics into their cases alongside typically used materials such as sand and rocks. Other caddisfly species use flexible material, such leaves, to build their case, which they first fragment to the correct size with their mandibles. This study explored the ability of *Agrypnia pagetana* larvae to fragment and utilize flexible plastic film to build portable cases and observed whether their interaction with plastic could be a source of biological microplastic creation. Larvae were removed from their original cases and incubated with 6x3.4mm pieces of microbially-conditioned polylactic acid (PLA) film and oak leaf. One treatment (T1) was given just enough material to build a new case (13 plastic, 13 leaf), whilst a second treatment (T2) was given enough plastic and enough leaf to build a case out of only one material (26 plastic, 26 leaf). After six days, the amount of leaf and plastic used in cases was analysed; exposure water was filtered to 0.2 μm and imaged under a microscope to quantify the presence of microplastic fragments and FTIR was used to confirm the polymer identify of observed fragments. All larvae had constructed new cases after six days. In T1, all twelve larvae incorporated PLA into their new case. In T2, eleven of the twelve larvae had incorporated PLA into their case, despite nine of these larvae still having unused leaf material remaining in the jar. Plastic fragmentation was evident, with 53% of PLA pieces in T1 and 43% of PLA pieces in T2 being fragmented. PLA microfragments were found in exposure water from both treatments, with up to 924 microfragments (45 - 928 μm) created by just one larvae over six days. This study found that *A. pagetana* larvae fragmented and used PLA film to build new cases - which led to microplastic release to the surrounding water. Plastic was incorporated into cases even when natural material was replete, indicating the likelihood of this behaviour to occur in natural habitats. These findings demonstrate the need

to consider the role of biological fragmentation in plastic degradation and should be considered when developing models to predict the fate and behaviour of microplastics in the environment.

3.10.P-We068 Release of Micro- and NanoPlastics From Single-Use and Reusable Facemasks

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Release of micro- and nanoplastics from single-use and reusable facemasks Yuyue Huang (presenter), Elvis Genbo Xu Department of Biology, University of Southern Denmark, Odense 5230, Denmark Presentation type: **Poster** Face masks helped prevent the spread of coronavirus and mass masking is recommended by almost all health groups and countries to control the COVID-19 pandemic. The massive production, use, and inappropriate disposal of plastic face masks result in new concerns of the plastic problem. The single-use masks ended in the natural environments may fragment into micro- and nanoplastics (MNPs) that are known hazardous to environmental organisms. Some new reusable face masks, therefore, are made and recommended but they may also release MNPs during sterilization and washing. In this study, we investigated the release of MNPs from both single-use and reusable face masks under both environmental aqueous and sedimentary conditions, as well as during sterilization, hand, and machine washing procedures. The efficiency and recovery of different MNP extraction/separation methods were also compared, including chemical digestion (acids, alkalis, and Fenton's reagent), enzymatic digestion (Proteinase-K and cellulose), density floatation (NaCl, ZnCl₂, and NaI), centrifugation, and sequential filtrations. This study provides important data on face masks as a new MNP source and it is urgent to recognize this environmental threat, particularly under the circumstance of facing new challenges of the coronavirus variants. **Key words:** microplastics; disposals masks; sediments; soils

3.10.P-We069 Release of Microplastics From a Newly Developed Bio-Based Composite After Ultraviolet Irradiation

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The dependence on petroleum-based polymers such as polypropylene (PP) has led to a series of environmental issues, including the persistence of microplastic (MP), i.e. plastic particles smaller than 5 mm in diameter, in the global ocean. Polymers made from a natural-sourced feedstock, like polylactic acid (PLA), known as bio-based polymers, are seen as more sustainable alternatives. However, our knowledge remains limited about their degradation and fate in the marine environment. Studies have provided evidence of the release of MP from larger debris under Ultraviolet (UV) radiation in laboratory conditions. However, the most direct and quantitative evidence of MP formation, i.e. observation, identification and enumeration of MPs formed after UV radiation, is limited. Indeed, only a few studies have assessed the disintegration of bio-based polymers and their capacity to form MPs. As part of the Interreg 2 Seas Mers Zeeën project SeaBioComp (seabiocomp.eu), we aim to compare, quantify and characterise the MP formation of a newly developed bio-based composite (i.e. bio-based polymers integrated with synthetic or natural fibres) and a reference petroleum-based polymer during their degradation under UV radiation. To do so, we exposed 3D printed cylinders (d=h=1cm) of self-reinforced PLA (SR-PLA) and PP respectively, immersed in natural seawater, to accelerated UV radiation for up to 1368h, simulating about 18 months of natural solar exposure in central Europe. Dark controls (i.e. sealed from the UV) were incubated in the same conditions also for 1,368h. To identify, characterise and quantify the formed MPs, we used a combination of fluorescent microscopy, infrared technology (FT-IR) and image analysis. We found that 1,368h UV exposure accelerated the MP formation of PP samples but not SR-PLA samples, suggesting that the newly developed bio-based composite SR-PLA is more resistant to releasing MPs than the reference petroleum-based polymer. We anticipate that our results will contribute to assessing the sustainability of future bio-based polymers and composites applications and to supporting a transition process to more sustainable plastic materials.

3.10.P-We070 Response to Microplastic Exposure: An Exploration Into the Sea Urchin Immune Cell Proteome

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Nowadays, it is undeniable that marine organisms are interacting with plastic debris with serious consequences on organism and population health mostly based on size lower than 5 mm defined as microplastics (MPs). Despite the growing attention on MPs pollution, there are still several scientific issues to be addressed in understanding MPs toxicity especially related to the molecular mechanisms and the cellular processes that are activated in response to MPs exposure. Translating this knowledge-gap on the biological impacts of MPs on one of the key species of the Mediterranean Sea, the *Paracentrotus lividus*, this study aims to explore for the first time the sea urchin immune cells profile combined to their proteome upon *in vivo* exposure to different concentration of polystyrene-microplastics (micro-PS). In detail, adult specimens of the sea urchin *P. lividus* were exposed to different concentration of fluorescent micro-PS (45 µm) (0, 10, 50, 1000, 10⁴ particles/L) for 72h. Already after 24h of micro-PS exposure, immune cells showed a significant increase at highest concentration tested (10⁴ particles/L), while the low micro-PS concentrations tested (10 and 50 particles/L) reported no significant differences. Moreover, coelomocytes showed an increase in both intracellular reactive oxygen and nitrogen species levels (ROS and RNS) at 24h and 48h, compared to the control. In the case of the RNS, the increase is maintained after 72h of exposure also to 10 and 1000 particles/L. Proteomics analysis revealed the presence of 2060 control group proteins. By comparing these proteins to the total proteins found in the treatments, 69 unique proteins were identified for 10 part/L, 75 for 50 part/L, 93 for 1000 part/L and 82 for 10⁴ part/L. Among these unique proteins, only the 8% of proteins (n=13) are shared by all four concentration tested simultaneously and mostly involved in cytoskeletal organization, intracellular membrane trafficking as well as protein metabolism. Overall, these findings provide new insights in

understanding the mode of action of MPs in the sea urchin immune cells at the molecular and cellular level. The combination of “-omics” approaches and ecophysiological techniques affords a more holistic understanding of the potential impacts of microplastics providing new perspectives for environmental risk assessment purposes.

3.10.P-We071 Finding the Culprit. Point-Source Tracking of Microplastic Concentrations in a Sewer Network

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1. Introduction Plastic has spread rapidly after its discovery and has become essential to human life. While plastic production has increased, plastic waste management lagged behind, causing an accumulation of plastic debris in the environment. Some of the plastic enters the environment as small particles < 5 mm, defined as microplastics (MP). Urban areas have been identified as a prominent source of MP release to the environment. Stormwater, wastewater and combined sewer overflows can be major sources of MP. In developed countries, wastewater from households, institutions, and industries is usually collected by sewer systems, then gets treated at wastewater treatment plants. However, 48% of global wastewater produced is released to the environment untreated. Our focus is to investigate the distribution of MP in wastewater produced from the different activities before entering the wastewater treatment plants. In the current study, we analyzed wastewater coming from several domains in Toulon, France. **2. Materials and methods** The wastewater samples were taken with an autosampler over 24 hours from manholes receiving sewage from the different activities as mentioned previously. The samples underwent extensive treatment using SDS, H₂O₂, three types of enzymes, and density separation before analysis. Subsequently, the concentrated samples were analyzed with a state-of-the-art FPA- μ FTIR instrument in transmission mode, where MPs were quantified in terms of particle number and mass, polymer composition, size distribution in the size range 10 – 500 μ m. This technology was combined with an automated analysis of the vast amounts of data that such analysis produces. **3. Results and discussion** The results show that industrial laundry facilities release considerably more MP than the rest of the investigated areas. The second and third largest concentrations were found at Car garage and Domestic. The distribution of the polymer types in each sampling spot was also investigated. More than 80% of the MPs in the wastewater originating from the laundry facility were identified as Polyester. **4. Conclusion** In this study, we showed significant differences between different types of wastewaters in a sewer network, a knowledge that is important when assessing MP pollution and designing protective measures.

3.10.P-We072 Microplastics in a Highway Stormwater Retention Pond

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It is known that different pollutants accumulate in stormwater retention ponds, including microplastics (MP). When investigating MP in such retention ponds car tyre rubber is often overlooked, even though there is a big potential for accumulation of these, especially if the stormwater retention ponds are located next to highways, large roads, or urban areas. This also results in insufficient knowledge regarding the accumulation of rubbers in these ponds. This study aims to quantify the mass of car tyre rubber in a stormwater retention pond using pyrolysis-GC/MS. Here the rubber content in the sediments are investigated at different depths namely, 0-3 cm and 3-6 cm. The objective is to increase the knowledge on the fate of car tyre rubber in such pond. In total 6 locations were sampled in a highway stormwater retention pond located at Låsby in Denmark using sediment corers. For each location two samples were collected one from the top layer (0-3 cm) and one from the middle layer (3-6 cm), ending up with a total of 12 sediment samples. To extract the rubber, each sample went through extensive sample preparation, including processes such as oxidation, density separation, and enzymatic treatment. After sample preparation the mass of rubber was quantified using pyrolysis-GC/MS with 4-vinylcyclohexene as the indicator product. Rubber was detected at all 6 sample locations in both the top and middle layer of the sediments. The concentrations were however varying. Both the lowest and highest concentration of rubber was measured in the top layer of the sediment, which indicates significant variance in the rubber concentration. This is also valid when comparing the top and middle layer as concentration between 35 μ g/g and 1084 μ g/g and 81 μ g/g and 997 μ g/g were measured in the top and middle layer, respectively. Even though there was substantial variation in the distribution of rubber in the stormwater retention pond, the levels were generally quite high, i.e. up to 0.1% of the sediment dry weight was constituted of car tyre rubber.

3.10.P-We073 Exposure of Microplastics at Levels Relevant for Human Health: Acute and Chronic Cytotoxicity and Cellular Localization of Polystyrene Microparticles in Four Human Cell Lines

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The ubiquity of microplastics (MPs) in the environment raises concerns on human health through potential exposure via air pollution, the food chain and drinking water. However, little is known about the effects of MPs on human health at realistic exposure levels. Herein, the chronic and acute effects of MPs at human realistic exposure levels on four types of human cell lines derived from colon (Caco-2), liver (HepG2) and lung (A549 and BEAS-2B) were investigated. The undifferentiated and differentiated cells involved the most representative exposure routes (inhalation, ingestion, and physiological transport through the liver by the portal vein) were exposed to 2- μ m fluorescent polystyrene (PS) microspheres (10³-10⁸ particles/L) for 48 h and 12 d. The measurements of flow cytometry and laser confocal microscopy showed that PS could be internalized in cell membrane and even in the nuclei. PS did not cause any significant toxic impacts on mitochondrial membrane potential and cell viability, but

induced reactive oxygen species production in undifferentiated A549 and Caco-2 cells upon short exposure, and differentiated A549 cells upon short and chronic exposure. The adenocarcinoma A549 cells from the lung seems to be more sensitive to PS exposure than normal bronchial BEAS-2B cells. Differentiated cells show a higher tolerance to PS than undifferentiated cells under the same exposure conditions. This research is a first step towards more relevant exposure studies to assess risks associated with ingestion, inhalation and internal transport of MPs in the human body.

3.10.P-We074 Retention of Microplastics and Tire Wear Particles in Stormwater Retention Ponds

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Stormwater runoff from roads and urban areas can contain a multitude of different pollutants. Stormwater detention ponds are used to mitigate the possible detrimental effect these might cause when released to the environment. However, their effects with regards to emerging pollutants such as microplastics and tire wear particles are not so well-understood. The objective of this study was to investigate the retention efficiency for microplastics and tire wear particles in stormwater retention ponds. To this end sediment and effluent samples from four different Danish stormwater retention ponds were analyzed. The sediment samples were collected as pooled sample from several locations in each pond, while the effluent samples were collected by filtration during three separate sampling campaigns using a specialized filtration system capable of collecting particulates down to 10 µm. This way between 2.4 – 3.2 m³ were sampled at each site. Prior to analysis the samples were subjected to a multi-step sample preparation consisting of oxidation, enzymatic digestion, and density separation with sodium polytungstate solution at 1.9 g/cm³ in an effort to remove organics and minerals while preserving polymers. Then triplicate subsamples were analyzed by FPA-FTIR imaging (Focal Plane Array-based Fourier Transform Infrared spectroscopy) and processed using siMPLE, a software developed for automated detection of microplastic. Subsequently, other subsamples were analyzed by Pyrolysis GC-MS (Gas Chromatography Mass Spectrometry) for identification of tire wear particles. Microplastics were found in all samples and more than 16,000 MP particles had their size, polymeric composition and mass measured. Across all ponds the average retention efficiency in terms of mass for MP were 83 %. Tire wear particles were identified in all sediments but in only one of the effluent samples, which gave an average retention efficiency of 95 % across all ponds. These results indicate how stormwater retention ponds can act as a sink for microplastic and tire wear particles and mitigate the spreading from urban areas.

3.10.P-We075 Temporal Fluctuations in Microplastic Concentrations Downstream From Sewage Treatment Works: A High-Resolution Study

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Microplastics (< 5mm) have been recovered from all environmental compartments, from deep-sea to the Arctic. It is hypothesized that wastewater treatment plants (WWTP) and sewage treatment works (STW) act as point sources for microplastics in freshwater systems. Some recent literature has estimated that discharge from German WWTPs contains up to 7 ? 10¹² microplastics (in size range of 10 and 5000 µm) annually (Schmidt et al., 2020) However, these calculations often are based on snap-shot sampling (i.e., single or limited time points) that may not account for potential changes in release patterns that may have large fluctuations in a course of a single day. There is limited understanding on whether microplastic abundance fluctuates in the course of a single day, or whether they stay broadly the same. This study aims to assess the validity of snapshot sampling and evaluate typical variability of microplastic abundance in a river system with known point source. To address this question, a four-day high-frequency sampling campaign was carried out downstream from a medium-sized Barston STW, located near Birmingham, UK. Sampling comprised two weekdays (Tuesday and Wednesday, 15th & 16th June 2021) and two weekend days (Saturday and Sunday 19th & 20th June 2021). Each day, 50 L surface water samples were collected in triplicates at one-hour intervals covering a twelve-hour period on each sampling day. Flow velocity was obtained using a Sensa-RC2 (Aqua Data Services Ltd) electromagnetic velocity meter water velocity meter, to aid in discharge and mass-flux calculations. Samples (lower size limit 64 µm) underwent organic matter digestion using wet peroxide oxidation with Fe²⁺(aq), and were subsequently stained with Nile Red (5µg mL⁻¹) in deionized water. Fluorescence microscopy in combination with Raman spectroscopy was applied to identify and quantify microplastics shape, size and polymer type. Initial results suggest relatively low hourly and daily variability in the overall concentration of microplastics in surface water despite significant changes in river height (~30 cm) and discharge (0.55 m³ s⁻¹ to 2.13 m³ s⁻¹) that were linked to effluent releases over the day. Future work will compare polymer composition and any possible fluctuations observed, and integrate the intensive 4-day sampling into a longer-term monthly sampling over a 1 year period. Schmidt, C., Kumar, R., Yang, S. and Büttner, O., 2020. Microplastic particle emission from wastewater treatment plant effluents into river networks in Germany: Loads, spatial patterns of concentrations and potential toxicity. *Science of The Total Environment*, 737: 139544.

3.10.P-We076 Effects of Microplastics Containing Additives on Blue Mussel (*Mytilus edulis*): Study of the Ageing and the Sorption of PAHs As Influencing Factors

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The effects of microplastics at environmental levels have yet to be fully examined taking into account the factors of ageing and sorption of some ubiquitous environmental contaminants, such as polycyclic aromatic hydrocarbons (PAHs). Current hypotheses indicate that microplastics which have been degraded by ultra-violet (UV) radiation and/or sorbing PAHs could have a greater

impact on the organism, for example by the transfer of additional contaminants to the organism. This study aimed to assess the influence of these factors on the sentinel species, *Mytilus edulis*, combining biochemical marker analyses and a lipidomic approach. The microplastics used in the experiment were polyethylene microplastic beads (MP). Then, some of them were irradiated for 1000 h in an accelerated ageing chamber (AMP). To simulate PAH sorption, raw microplastics and irradiated microplastics were added to a solution of 4 PAHs (MP-PAH and AMP-PAH respectively). The contamination of tanks was done with the 4 types of microplastics at 10 µg/L, with a treatment without contamination (Control). Fifteen individuals were exposed for 8 days in 15-L tanks filled with artificial seawater per treatment and in triplicates. The potential of microplastics to induce neurotoxicity was determined by Acetylcholinesterase (AChE) measures. Superoxide dismutase (SOD), catalase (CAT), and glutathion S-transferase (GST) served to monitor oxidative stress. These biomarkers were measured on gills, digestive gland and mantle after dissection. Acid Phosphatase (AcP) was used as biomarkers for immunotoxicity and measured in hemolymph. Finally, lipidomic analyses were performed using flow injection analysis coupled to high resolution mass spectrometry (FIA-HRMS) on lipid extracts of digestive glands. All the experiments were performed from 5 individuals per tank, *i.e.* 15 par condition. The combination of artificial ageing and sorption of PAH factors for microplastic exposures showed effects on the blue mussel after 8 days of exposure at environmental doses (10 µg/L) with the detection of oxidative stress and neurotoxicity which were organ dependent. The mantle, not usually analyzed in ecotoxicological studies, seemed to be an interesting organ to monitor the biochemical effects of microplastics. None marked influence of either the ageing or the PAH sorption was highlighted through the lipidomics. Biochemical and lipidomic approaches are complementary tools in the environmental risk assessment.

3.10.P-We077 Influence of Microplastics on Rates of Potential Nitrification and Denitrification in Floating Treatment Wetland Systems

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Microplastics, plastic particles smaller than 5 mm, are a contaminant of emerging concern due to their potential for detrimental environmental and human health effects and persistence in the environment. Documented in an increasing array of environments, microplastics are commonly found in sources that traditionally contain high levels of other contaminants, such as urban and agricultural runoff. Floating treatment wetlands are a method of remediation for nutrient pollutants, especially nitrates and phosphates. As microplastics are also found in similar sources as those contributing to nutrient pollution, and microplastics have been shown to impact nitrogen transformation processes in wastewater treatment plants, microplastic pollution may impact similar processes in remediation efforts such as floating treatment wetlands. To evaluate the potential impact microplastics may have on nitrogen transformation in these environments, root samples from an established floating treatment wetland were collected and used to inoculate microcosms to study nitrification and denitrification processes. Microplastic treatments included polyethylene and polystyrene microspheres at two diameters (30 µm and 200 µm) for three concentrations (10 mg/L, 100 mg/L, and 1000 mg/L). Nitrification microcosms were observed in aerobic conditions over a 24 hour period and denitrification microcosms flushed with nitrogen gas to encourage anoxic conditions were monitored over a week. Nitrate and nitrite production/consumption was analyzed using a microplate method to calculate potential nitrification and denitrification rates. A preliminary potential denitrification experiment using 1-4 µm polyethylene microbeads found there was not a significant difference in potential denitrification rate between microplastic concentrations (10 mg/L and 100 mg/L) nor between the added microplastic treatment and root controls. Preliminary results from nitrification microcosms show similar results where the addition of microplastics do not impact potential nitrification rates. Further research to observe the impacts of weathering and chemical additives would give a better understanding on how microplastics in the environments may interact with microorganisms and influence nitrogen transformation in the system.

3.10.P-We078 Plastic Content and Additive Distribution in Wastewater Treatment Plant Effluent in Oslo (Norway)

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Microplastics have been the subject of increasing focus over the last decade since they have been found in virtually all waters and oceans around the globe. While rivers are assumed to be a major contributor of microplastic pollution to the marine environment, treated and untreated wastewater effluents and stormwaters are expected to be a significant emission source of not only microplastic particles but also of the less studied nano-size plastic to freshwaters. Chemical additives are generally added to plastics during production to enhance certain properties, e.g. flexibility, flame retardancy, or lifetime. The leaching capability of additives such as plasticisers, flame retardants, UV stabilisers or residual curing agents is dependent on the type of plastic and the chemical agent itself. The release of chemical additives to and fate in the environment is linked to the fate of plastic (emission, particle size or breakdown). It also remains totally unclear whether microplastics release to the environment currently provides an unaccounted-for pathway for the release of chemical additives to the aquatic environment. The objective of this study was to assess the distribution of selected additives (flame retardants, plasticisers and UV filters) in wastewater effluents in Oslo (Norway). Suspended particulate matter was sampled from the effluent using continuous flow centrifugation. The plastic content was measured with pyrolysis GC/MS while different extraction and sample cleanup procedures were applied to extract additives. Total additive concentrations were obtained with Soxhlet extraction while silicone rubber extractions were applied to estimate the easily desorbable additive concentrations. Other sample treatment options tested included KOH and H₂O₂ treatment prior to Soxhlet extraction. Freely dissolved additive concentrations were obtained by passive sampling in situ in the effluent itself. Results of this work include estimates of plastic content of the effluent and of the distribution of selected additives in the suspended matter of the effluent.

3.10.P-We079 Early Stages of Biofilm Formation on Bio-Based Microplastics in a Reservoir

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Bio-based plastics, produced from renewable biomass sources, are currently increasing in products and applications, including high-density polyethylene (HDPE), polylactic acid (PLA), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). However, their biodegradability and environmental fate are not fully understood, especially microplastics. Therefore, we performed an *in-situ* study in a freshwater reservoir in the winter season. Three pristine microplastics materials (HDPE, PLA and PHBV) were separately placed into cellulose dialysis bags and anchored in a cage in the inflow part of the reservoir. Initial stage of biofilm formation on plastics was investigated using 16S rRNA sequencing to identify rare and more abundant bacterial taxa (> 5% of total biomass) were distinguished. After two months, bacterial and algal abundance on plastics increased in the order of PHBV > HDPE > PLA, while fungal abundance was highest on HDPE > PHBV > PLA. The biofilm composition showed that dominant bacterial populations were irrespective of plastic types, while rare taxa were significantly diverse. The dominant taxa belonging to *Caulobacteraceae/Asticcacaulis*, *Cellvibrinaceae/Cellvibrio*, and unclassified *Oxalobacteraceae* were unchanged on all plastics during the study. Within the rare families, *Bacteriovoracaceae*, *Crocinitomicaceae*, *Oxalobacteraceae*, *Sphingobacteriaceae* increased on all plastics after 2 months. Overall, rare taxa specific to each plastic type were uncultured bacteria on HDPE, *Cytophagaceae* on PLA, and *Sphingobacteriaceae* on PHBV.

3.10.P-We080 Fate of Microplastics in Background Headwater Lake Catchments, Ontario, Canada, Using a Microplastic Particle Balance Approach

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Microplastics are pervasive contaminants of concern, but the fate of microplastic pollution in headwater lakes, especially in background regions, remains relatively unknown. Few studies have quantified or synthesized the inputs, outputs, and pathways of microplastics to lakes at the watershed or catchment level. In this study, the flux of microplastics (MP) were quantified over a 12-month period for three background headwater lake catchments in Muskoka-Haliburton, Ontario, Canada. A microplastic particle balance approach was used, incorporating inputs from atmospheric deposition and inflows against outflows and sedimentation to lakes. Lake samples were collected 1-metre below the surface from each of the three study lakes and stream samples were collected from 17 gauged inflows and outflows as grab samples from the point of controlled discharge. Duplicate sediment cores were collected from the deepest point of the lake and deposition samples were collected from 3 stations using bulk precipitation collectors. The results showed that atmospheric deposition had the highest daily microplastic flux rate (3.84–8.04 MP/m²/day), between 1.5 and 4 times greater than the flux of microplastics in the inflow streams (2.14–2.34 MP/m²/day). This suggests that the atmosphere is the dominant source of microplastics to lakes in background regions, accounting for all of the inflowing microplastics to the study sites. Of the microplastics deposited on the catchment from the atmosphere, 41–73% were retained in the terrestrial area. Furthermore, a large fraction of the microplastics in the inflow streams were retained in the lakes (30–45%). This suggests that lakes are a reservoir for microplastics; in the current study the residence time for microplastics ranged from 3.15 to 7.70 years. Fibres were the dominant shape identified in atmospheric deposition, stream water and lake water; however, in lake sediment, there was a higher proportion of fragments. For all sample media, polyethylene terephthalate (20%) was the dominant polymer identified followed by polypropylene (18%) and polyamide (14%).

3.10.P-We081 Detection of Plastics in Soil - Prevalence, Origins and Effects

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Soil acts as a final sink for pollutants. Microplastics from different sources such as plastic mulching, littering, compost, sewage sludge, sedimentary deposition, and tyre abrasion are expected to be found in soil. However, representative and comprehensive information is missing on the sources, transport, and fate. Therefore, a reliable analysis method for microplastics in soils needs to be developed. [1]The work presented here describes the development of a procedure for microplastics analysis in soil. A representative sampling based on the on-site conditions and a sample preparation method were established including a drying step, the separation of microplastic particles > 1 mm, and a density separation for particles < 1 mm. The detection of the large microplastic particles (> 1 mm) was conducted with Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR-FTIR), while Thermal Extraction Desorption - Gas Chromatography / Mass Spectrometry (TED-GC/MS) [2] was applied for particles < 1 mm, gaining information on the type of polymer and mass fraction. Based on the established method, 14 environmental soil samples with different exposure to microplastics from agriculture, industrial sites, roads, and floods were investigated. High contents of small microplastic particles (< 1 mm) were discovered in the soil samples exposed to plastic mulching, fertilization with sewage sludge or compost (0 – 115 mg/kg). On average, microplastic contents detected in soil samples taken from a construction site and an inner-city lake were higher (13 – 238 mg/kg). As expected, microplastic contents in soil sampled in proximity to roads was more pronounced in the upper soil layers. In contrast, very remote sampling sites, believed to be uncontaminated, did not lead to the detection of microplastics by TED-GC/MS. In a proof of concept experiment several *in vivo* and *in vitro* ecotoxicological tests were applied to evaluate the effect of microplastics (tyre abrasion, polystyrene containing

hexabromocyclododecane) in natural soils. In summary, while no effects of the examined probes could be detected on higher levels of biological organization after exposures to earthworm *E. andrei*, significant changes in several oxidative stress related biomarkers were observed. [1] Moßler, Julia N., et al. *Environmental science & technology* 54.4 (2020): 2078-2090. [2] Dümichen, Erik, et al. *Journal of Chromatography A* 1592 (2019): 133-142.

3.10.P-We082 Transfer of Microplastics From Aquatic to Terrestrial Environments

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Microplastics are important emerging contaminants in marine, aquatic, and terrestrial environments. As microplastic pollution increases, it is important to understand their occurrence, fate, and transport in these environments. While the trophic transfer of microplastics within ecosystems has been studied, we lack empirical evidence of the trophic transfer of microplastics across ecosystems. This study aims to characterize a pathway of microplastic movement from aquatic to terrestrial ecosystems, using *Tachycineta bicolor* (i.e., tree swallows) as a model. Here, we analyze samples of water, sediment, insects, and lower digestive tracts of *T. bicolor* nestlings for microplastics from three locations along the St. Louis River in Minnesota and three locations in Wisconsin. In each state, we examined urban, suburban, and rural sites. Modified versions of the NOAA Marine Debris Program's Laboratory Methods for the Analysis of Microplastics in the Marine Environment were applied to remove as much organic material from the water and sediment samples as possible to better detect plastic particles without significantly altering them. Plastic polymers were identified with Fourier-Transform Infrared Spectroscopy (FTIR). Overall, the results show that microplastics were present in the lower GI tracts of in nestling tree swallows, water, and aquatic insects. Although the concentrations were not significantly different across the six sites, tree swallows collected from urban sites appeared to have higher number of microplastics in their GI tracts than tree swallows from rural sites. Interestingly, concentrations of microplastics in tree swallows are strongly correlated with microplastic concentrations in aquatic insects-Odonatans but not Dipterans. These results suggest that Odonata could be the main food source for nestling tree swallow and carried microplastics to the birds- a mechanism of microplastic transfer back to terrestrial ecosystem from aquatic ecosystem. To our knowledge, this scientific evidence for microplastics transfer across ecosystems was observed for the first time.

3.10.P-We083 Evidence for Very High Microplastic Contamination in Amphibian Breeding Pools and First Data Indicating the Potential for Harmful Effects on Wild Tadpoles (Common Toad, *Bufo bufo*)

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Microplastics (MP) have been widely reported in surface water, sediment and biota sampled from aquatic environments. However, relatively few data exist on MP contamination of small freshwaters (SFWs) such as ponds, puddles and ditches, which are likely to harbour high levels due to their low volumes combined with probable high exposure levels. Amphibians are one of the most common vertebrates to inhabit SFWs and are undergoing catastrophic decline globally. We investigated MP pollution of SFWs and common toads (*Bufo bufo*) under different land use characterisations. Water and sediment samples were collected from reference (R: $n = 3$), urban (U: $n = 3$) and agricultural (A: $n = 3$) ponds at 4 timepoints in the Spring (15th March - 9th June). Where found (1 x R, 1 x U, 2 x A), 20 tadpoles were collected *per site* on visits 3 and 4. The levels of MPs found across all site characterisations ranged between 0–30 items/L⁻¹ in water and 12–1484 items/kg⁻¹ in sediments, exceeding levels found in coastal environments globally (~ 0.002 items/L⁻¹, ~ 60 items/kg⁻¹). MP abundance, shape and size did not differ between site characterisations, but some differences in polymer type were observed. For example, styrene-butadiene rubber ($n = 1$ particles) was only found in urban sediments, whereas polyvinyl chloride ($n = 2$), ethylene-vinyl acetate ($n = 2$), and epoxy ($n = 1$) were only found in reference and agricultural surface waters. Tadpoles also contained MPs ranging between 0 – 163 items/g tissue⁻¹ (0 – 19 items/individual⁻¹) that were 10 - 100-fold higher than previously reported for other freshwater vertebrates. The vast majority of MPs were found in the gut (81.26 %, $n = 464$) compared to the liver (8.06 %, $n = 46$), brain (2.8 %, $n = 16$) and carcass (7.88 %, $n = 45$), suggesting dietary uptake. This is the first study to report MPs in the brain of a wild vertebrate. Across all sites, a significant negative correlation ($p < 0.01^{**}$) was found between tadpole MP abundance and weight ($R^2 = 0.1135$), total length ($R^2 = 0.1587$) and snout-vent length ($R^2 = 0.1805$), suggesting that MPs may be negatively impacting wild tadpole health. These results provide important insights into the extent of MP pollution in SFWs, which currently do not form part of routine water quality monitoring, as well as potential links between MP ingestion and health-endpoints in wild amphibians for the first time.

3.10.P-We084 Occurrence and Ecological Risks of Microplastics in the Amazon River

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The main objective of this study was to characterize the contribution of large cities of the Brazilian Amazon to MP exposure in the Amazon river; to characterize their particle size, shape, and polymer distribution; as well as to assess their risks for freshwater organisms. For this, we analyzed the MP content of 40 water samples collected along a longitudinal gradient of more than 1500 km, which included urban streams and areas of the Amazon River with relatively low anthropogenic impact. MPs were found in all samples, with concentrations varying from 5 MPs/m³ to 74550 MPs/m³. Overall, the total concentration of MPs was higher in

surface waters close to urban areas: streams near the city of Manaus had the highest, followed by those close to Belém, Macapá and Santarém, respectively. MP pollution was dominated by particles >300 µm, where fibres of polyester and fragments of polypropylene, polyethylene, and polystyrene were most abundant. The measured MP concentrations were compared with available threshold concentrations for aquatic organisms (PNECs). MP concentrations were lower than the available PNECs in 93% of the samples. However, three samples taken near Manaus had concentrations above at least one of the available PNECs. This study shows that MPs are ubiquitous contaminants in Amazonian surface waters and that the release of untreated urban wastewaters clearly contributes to increased exposure levels. Our risk assessment shows that ecological impacts may be expected in pollution hotspots near densely populated areas, such as the city of Manaus, which has very limited wastewater treatment. Further work includes the calculation of MP emission by the different urban areas, the total MP loads into the ocean, as well as the refinement of risk calculations with species sensitivity distributions.

3.10.P-We085 Lateral Distribution of Surface-Floating (Micro) Plastic Particles in the North Pacific Ocean

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The mass of globally produced plastic is constantly increasing and nearly reached 370 million tons in 2019. Among other factors, insufficient waste management systems and littering lead to the pollution of the marine environment with plastic particles. In the environment, plastic particles are affected by mechanical, photochemical and biological processes which lead to their weathering and the formation of even smaller particles. Assuming a business as usual scenario, the mass of plastic in the oceans continues to increase and will reach more than 250 million tons by 2025. In the oceans, it accumulates in specific regions due to the influence of ocean currents and winds. One of the biggest known accumulation zones is the so-called Great Pacific Garbage Patch between California and Hawaii. The aim of this study is to determine the lateral distribution of plastic particles floating at the sea surface in the Pacific Ocean. Therefore 10 samples were collected during cruise SO268/3 of the German research vessel SONNE between Vancouver and Singapore from May to July 2019 using a Neuston catamaran with a mesh size of 330 µm. Plastic particles >500 µm were submitted to sieving and enzymatic digestion and afterwards analyzed by ATR-FT-IR spectroscopy. Smaller particles were analyzed by FT-IR imaging in transmission mode after deposition on Anodic filters. Here we present our results for the distribution of surface-floating plastic particles >330 µm. We found plastic particles at every sampling station with concentrations that were extrapolated to 10.000 to 173.000 particles per km². Furthermore we were able to identify two regions with highly elevated concentrations of floating plastic particles – the North Pacific Subtropical Gyre and an area belonging to the Papahānaumokuākea Marine National Monument. These results are consistent with litter survey and modeling data.

3.10.P-We086 Chemical Additives in Weathered Microplastic in the Marine Environment - Occurrence and Risk

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A Nordic project identified and measured residues of chemical additives in two plastic materials made from the commonly used polymer types polyurethane (PUR) and polyvinyl chloride (PVC). Measurements were performed in applied (new) plastic, and in plastic exposed to weathering for four months in the marine environment. To simulate some degree of weathering the plastic was granulated into particles in micro-meter size (microplastic) (250 µm – 710 µm), and exposed in water-permeable bags in the Samnangerfjord near Bergen from June to October 2020. A suspect list of known and new chemical additives was constructed for PUR and PVC. Targeted chemical analysis with GC-Orbitrap was performed for identification of the suspect chemicals in the plastic particles before and after weathering. For the identified chemicals, GC-MS analysis was performed for quantification. Four plasticizers were detected in PUR, and three in PVC, before and after weathering: Dibutyl adipate, N-butylbenzenesulphonamide, dibutyl phthalate, and di-2-ethylhexyl phthalate (DEHP). Maximum concentrations were 1587 ng/g plastic before weathering, and 946 µg/g plastic after weathering. Two detected additives were a heat stabilizer (triphenyl phosphite) and an antioxidant (octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate), identified both in PUR and PVC before and after weathering. Measured concentrations were used to calculate PECs for the pelagic environment (copepods and cod), and for secondary poisoning of fulmar. PNECs and risk quotients (RQ=PEC/PNEC) were derived. Additionally, non-target screening with GC-Orbitrap was done to identify chemicals with certain structures found in the NIST library and MassBank database. Proper filters were applied to further identify phthalates, polyaromatic hydrocarbons (PAHs), chlorinated or brominated compounds, and aromatic nitro compounds, which can be considered as representatives of plasticizers, impurities in PVC, flame retardants, and biocides, respectively. Sixteen chemicals were identified with non-target screening. A PAH identified in PVC both before and after weathering, i.e. 2,6-Diisopropyl-naphthalene, used as plant growth retardant and agrochemical, is classified as very toxic to aquatic life with long lasting effects. A chlorinated compound identified in PUR both before and after weathering, i.e. 2-Propanol, 1-chloro-, phosphate (3:1), used as flame retardant, is classified as suspected of damaging fertility or the unborn child.

3.10.P-We087 Effect of Polyethylene Microplastic (MPs) and Adsorbed Organic Pollutants on *Zea mays* L.: Physiological and Transcriptomic Analysis

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Research on the environmental impact of plastics, especially on the effect of microplastics (MPs), has become a priority issue in

recent years, mainly in terrestrial ecosystems where there is a lack of studies. This work aims to assess the impact of three types of polyethylene MPs: white microbeads (W), blue microbeads (B) and fluorescent blue microbeads (FB), and their interactions with a mix of organic pollutants containing: ibuprofen (IB), sertraline (STR), amoxicillin (AMX) and simazine (SZ), on *Z. mays* L. plantlets developed under *in vitro* conditions. Murashige and Skoog (MS) plant culture media were prepared including one of each type of MPs, alone or combined with the organic pollutant mixture (MIX). Seeds of *Z. mays* were subjected to sterilization and sown in culture tubes. Seeds were incubated at 25 °C with a 16 h photoperiod and photosynthetic photon flux density (PPFD) of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from cool white fluorescent tubes. Ten seeds were sown per treatment, and a control without MPs or MIX was included in the analysis. After 2 weeks, germination and development were evaluated, and samples of root and leaf tissue were collected. The effect of the different treatments on the physiological characters of *Z. mays* plantlets was analysed through development parameters (root and leaf length), chlorophyll content, and oxidative stress level (ROS and RNS). In order to determine the molecular basis of the MPs and MPs-MIX complexes effects on maize development, a RNA-seq analysis was performed. The effect of the three MPs types on the maize plants development were significantly different, being the W microbeads the ones that caused the greatest inhibition in both root and leaf. Inhibition in root length was only detected when W MPs were combined with the MIX pollutants. Likewise, this last treatment supposed a significant difference in the chlorophyll content compared to the other MPs and MPs-MIX treatments. The three treatments in which MIX pollutants were adsorbed to MPs resulted in a significant increase of free radicals, compared to the control, in root samples, although this response was not observed in leaf samples. The negative effect of W microbeads also was detected in the transcriptomic analysis. Differences in gene expression were detected in the treatment with W MPs (without pollutants) when compared to the control treatment.

3.10.P-We088 New Environmental Monitoring Program; Microplastics in Norwegian Coastal Areas, Rivers, Lakes and Air (Mikronor)

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Norwegian Institute for Water Research (NIVA) have, on behalf of the Norwegian Environment Agency (NEA), started Norway's first microplastic (MP) monitoring program, together with Norwegian Institute for Air Research (NILU). The program, *Microplastics in Norwegian coastal areas, rivers, lakes and air (Mikronor)*, started sampling of different matrices in 2021 and will continue until 2024. Despite the continued challenges with MP assessments, including those on processing and analysis, there is a need to commence monitoring in order to address significant knowledge gaps. The primary aim is to provide information on levels and types of MPs in aquatic environments as well as in air. *Mikronor* will explore variations in MP concentrations between geographical areas and sample types, as well as possible sources. The project will gain important knowledge for MP monitoring and contribute towards national and international requirements, such as for the EU and OSPAR. *Mikronor* have received samples from a broad geographical span from large parts of the Norwegian environment (coastal sites, open marine waters, lakes and air) through pre-existing national monitoring programs that cover a range of different water bodies. The following matrices have been sampled in 2021: water samples collected using vertical plankton nets (~150 litres; 200 μm), subsurface seawater using a FerryBox system linked to the seawater intake of the cruise ship Color Fantasy (~5000 litres; 100, 200 and 500 μm meshes), high-volume surface pumps (1000 litres; 200 μm and 50 μm), urban runoff (~2 litres; 50 μm), effluents from wastewater treatment plants (~70 litres; 20 μm) and riverine water from one urban river (1000 litres; 200 μm and 50 μm). Soft bottom sediments samples (~600 ml; 50 μm) corresponding to the water sampling sites have also been collected, as well as biota: namely, marine mussels (Blue mussel, *Mytilus edulis*; 50 μm), freshwater mussels (duck mussels, *Anodonta anatina*; 50 μm) and different marine polychaetes (e.g. family Nephtys and Terebellidae; 50 μm). Both deposition and active air samples were collected for atmospheric MPs. The analysis of water samples will be conducted through a combination of scanning micro-fourier-transform infrared spectroscopy (μFTIR) and pyrolysis GC-MS, whilst the air samples will be analysed with a combination of RAMAN spectroscopy and pyrolysis GC-MS. Preliminary results and insights into the activities of the monitoring programme will be presented.

3.10.P-We089 Influence of Polyethylene Microplastics on Acute Toxicity of Tebuconazole on Different Earthworms

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Microplastic (MP) and pesticides are coexisting contaminants in agricultural soils nowadays. Deriving from degradation of introduced materials such as mulch films or secondary MP input through e.g. wind drift and sewage sludge application, microplastic is ubiquitous in soils of the agrarian landscape. As well as pesticides, MP can accumulate in the soil due to long degradation times and interact with pesticides. In this study we investigated the effects in the presence of microplastic on the toxicity of pesticides in worm species. However, since the standard worm in ecotoxicological testing, *Eisenia fetida*, is less ecologically relevant for agricultural toxicity assessment due to its natural habitat, we also included the species *Lumbricus terrestris*. The pesticide chosen for this study is the fungicide Folicur® (a.i. Tebuconazole), which is mainly used in rappt cultivation. The microplastic was derived from black polyethylene mulch film with a size range of 50 to 1000 μm and an application concentration of 150 mg/kg dw soil as not affecting, but realistic scenario. Investigation of MP influence was conducted in the acute toxicity test according to OECD 207 with *E.* and in the cast production test with advanced concentration range to test mortality with *L. terrestris*. The toxicity of Tebuconazole was determined with a LC50 of 112 mg/kg dw for *E. fetida* and 12 mg/kg dw for *L. terrestris*. For both tests the presence of MP lowered the acute toxicity of Tebuconazole, though not

significantly. The LC50 values in presence of MP were 121 mg/kg dw for *E. fetida* and 15 mg/kg dw for *L. terrestris*. We found significantly different pesticide toxicities on both species with *L. terrestris* being about 10 times more sensitive. We conclude that the influence of MP at the chosen concentrations of MP is low, but predictable and might be enhanced by higher concentrations of MP in soil. However, also the opposite effect was found in other studies with aggravated toxicity at MP co-exposition. Even though, MP might in this case have influence on the toxicity of Tebuconazole, the question remains, if effects like the Trojan-Horse-effect might lead to a higher selective toxicity long-term. Furthermore, the high difference in both worm species LC50 values opens the question how reasonable the testing with *E. fetida* for agricultural assessment is.

3.10.P-We090 The Impacts of Microplastics From Conventional and Biodegradable Mulching Films on Earthworm *Eisenia andrei*

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Mulching films are widely used in agriculture and horticulture to facilitate cultivation and to reduce the need of irrigation and the use of plant protection products. However, these plastic films can be prone to fragmentation when exposed to UV radiation and various physical strains in the field, and thus can act as sources of microplastics in the soil. As alternatives to conventional plastics, biodegradable plastics have been developed. In contrast to conventional mulching films, biodegradable mulching films are intended to be incorporated into the soil after use. However, in subarctic, cold climates decomposition can be slow and the material may accumulate in soil due to frequent use of biodegradable mulching films. Despite the release of particles of conventional and biodegradable plastics in agricultural soils due to use of mulching films, their potential impacts on soil biota are still poorly known. In this study, we investigated the effects of mulching film particles (< 1 mm) on earthworm survival, growth, reproduction and biochemical biomarkers. The earthworms were exposed to three different microplastic types: one conventional and two biodegradable plastics in concentrations of 0.1% and 1%. The experiments were performed twice: immediately after mixing the microplastics in the soil and approximately two months after the addition of the microplastics. In this presentation, the results of the experiments will be shown. This study is part of the project "Microplastics in agricultural soil – Sources, effects and reduction (MicaAgri)" funded by the Ministry of Agriculture and Forestry of Finland, from the Mäkelä Fund.

3.10.P-We091 Interaction of Unicellular Green Alga *Chlamydomonas Reindhardtii* With Biocompostable Plastic Bags

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A growing concern about fossil-based plastics pollution rises the fast growth of bio-based plastics as its alternative. The bio-based plastics derived from renewable biological sources, are believed to be more environmentally friendly. However, their biodegradation and fate in the environment are not fully understood. Here, we studied the initial phase of plastic biodegradation, which was an attachment of freshwater microalga, *Chlamydomonas reinhardtii* on three types of starch-based plastic bags with the main component of polyethylene terephthalate (PET), PET-C made from corn starch, PET-WPC made from wheat, potato and corn starch, and PET-CB made from cornstarch and beetroot. Algal growth rate, biofilm formation, and changes in plastic properties were evaluated. The plastic pieces (0.75 x 0.75 cm²) were added into the algal culture and incubated at laboratory temperature and low light density. The algal culture was then monitored by measurement of optical density at OD 680 nm and the biofilm formation on the plastics was observed under an epifluorescence microscope. The changes of plastic properties were analyzed by a scanning electron microscope (SEM) for morphology and a confocal microscope (CM) for surface roughness. After the first week, algal cells were already attached on the plastic surface forming colonies and biofilm. The highest cell density was found in samples with PET-WPC from wheat and potato starch following by PET-C and PET-CB from corn starch. In the second week, the SEM results showed a biodegradation signal by changes of plastic morphology compared to the pristine plastics. Moreover, plastic debris was detected in the supernatant. The experiment is continuing to obtain a complete picture of biointeraction of *C. reinhardtii* with plastics.

3.10.P-We092 Small microplastics(<100 Mm), Additives, and Plasticizers in Freshwater Filter Feeders: First Evidence of Ingestion in Black Flies

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Microplastic contamination has been an emerging issue in the last decade, and much data was produced to assess the distribution in environmental matrices. This contamination is more important as it is transferred to the food web and involves the biota. Along the food chain, the microplastic particles, in particular, the smallest (SMPs), and other contaminants present inside or adsorbed on, can be transferred throughout the trophic web. The current knowledge about the impacts of these pollutants on freshwater organisms is significantly incipient compared to marine organisms. Because of their relevant ecologic role, macroinvertebrates are commonly used in biomonitoring to assess freshwater's ecological conditions (European Water Framework Directive 2000/60/EC). Black flies (Diptera, Simuliidae) larvae are freshwater filter feeders; their special brush-like mouthpart collects tiny organisms and organic matter out of the water that flows through it, acting as a sieve or filter. They can also ingest microplastics when they get trapped in their mouthparts. They have a key role in watercourses ecology, making the filtered matter available for other invertebrates, amphibians, and fishes that feed on them. In this study, we analyze the MP uptake in 2 black flies species, *Simulium equinum* e *Simulium ornatum*, collected in Central Italy, to discuss the potential employment of this taxon as an indicator of microplastic pollution in freshwaters. The specimens were identified before the pretreatment. After chemical digestion to dissolve organic matter and purification, the samples were filtered on aluminum oxide filter membranes (0.2 µm, 47 mm

diameter). The analysis for the quantification and simultaneous polymer identification were performed via Micro-FTIR. Additives and plasticizers were also detected. The abundance of SMPs and additives in the studied species was relevant; this is the first report of SMPs intake on these organisms, to the best of our knowledge. These results are important to understand the mechanisms of plastic interaction with biota and identify the organisms to be used as bioindicators of plastic contamination.

3.10.P-We093 Microplastics in the Gastrointestinal Tracts of a Freshwater Chub (*Squalius vardarensis*) From an Eastern Mediterranean River

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Microplastic particles have been observed in the marine environment all over the world for three decades now, however, microplastics in freshwater ecosystems have attracted less attention until recently. Studies on microplastic pollution in inland waters typically concern lentic ecosystems, while research in lotic ecosystems is scarce. Our study area, Pinios River basin, can be described as a typical medium-sized European Mediterranean river; located in one of the most intensely cultivated agricultural area and densely inhabited regions in Greece. In total, 45 individuals from a native chub species (*Squalius vardarensis*) were used to assess the microplastic ingestion from the river. The results indicated high occurrence and abundance of microplastics (58 items) in the fish gastrointestinal tracts; almost two thirds of specimens (62.2%) contained microplastics. The average number of microplastics per specimen was 1.29 ± 0.2 (SE). Mean dimension length range of microplastics was $0.64 \mu\text{m} \pm 0.05$ (SE). The dominant type of the detected microplastics was fibers (66%), while fragments had a smaller contribution (34%) to the total amount of the ingested items. The most common colors of microplastics were blue and transparent (26% and 22%, respectively) followed by black and green (both with 19%). The major polymer types identified by FT-IR analysis were: 40% polyethylene (PE), 36% polyvinyl alcohol (PVA) and 21% polypropylene (PP); reflecting the fragmentation of larger litter from local sources, such as plastic sheets or plastic bags and pouches from industrial packaging or agrochemical products, and household goods. Surface runoff of the environment, via motorways and road network, could also be one more contributing factor to the reported microplastics. Our results suggest that microplastic pollution is well established in Pinios, along with other significant threats that the river is facing (e.g. groundwater over-exploitation, soil erosion, hydromorphological alterations, deforestation etc.) and could further augment through intensive anthropogenic activities such as extensive agrochemical use, semi-industrial activities and domestic sewage wastes from large cities in the area. Nevertheless, more research is needed to assess the microplastic contamination throughout the river basin, especially in reaches which are adjacent to large areas with mixed land use types, and whether the microplastic ingestion is dependent on fish species traits, and/or on plastic types.

3.10.P-We094 In Vitro and In Vivo Toxicity of Biobased & Biodegradable Polymers on Rainbow Trout Cell Lines and Juvenile Perch

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This study investigates the acute and chronic toxicity of biobased and biodegradable microplastics in freshwater fish species using in vivo and in vitro test systems. Our studies differentiate between polymer microparticles and the chemicals inherent to these materials. We used the microplastic particles in a size range of 90 – 150 μm , chemical extracts of larger particles and non-polymer natural Kaolin control particles. The toxicity of Polylactic acid (PLA) microplastic particles was assessed in juvenile perch (*Perca fluviatilis*) in a 6 months chronic food-exposure study, using several endpoints, life cycle parameters (growth, weight gain), metabolism, general stress response and behavior (locomotion, schooling and predator response). The cytotoxicity of chemicals associated with biobased and biodegradable microplastic polymers (PLA, PBS, PBAT, PBSA, P3HB and PLimC) was tested using chemical extracts on rainbow trout (*Oncorhynchus mykiss*) gill and liver cell lines. Cytotoxicity was studied on different cell viability and metabolic endpoints, such as lysosomal membrane stability, proliferation and enzymatic activities. Additionally, oxidative stress, gene expression and cell migration were investigated on the rainbow trout cells. Results of different measured endpoints indicate a cytotoxicity of the polymer P3HB on rainbow trout cell lines. The chronic exposure of juvenile perch to PLA particles had effects on growth and weight gain, as well as the Gonadosomatic index (GSI), compared to the control and natural particles (Kaolin) exposed fish. PLA-fed male perch showed a tendency to sexually mature faster than the other groups. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860720 (LimnoPlast ITN).

3.10.P-We095 Microplastic and Potential Harmful Trace Elements (PHTE) As Co-Pollution in Wild Brown Trout (*Salmo trutta*) in High Mountain Rivers

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Over the last few years, plastic has been defined as a new emerging pollutant found in every habitat across the globe. The fragmentation of large items of plastic litter into small particles is the main source of microplastics in natural environments. These tiny plastic particles can be easily transported, reaching all habitats, even the most pristine. Microplastics are an issue of great concern due to their size since they can interact between different ecosystem compartments and easily be incorporated into the trophic chain. Furthermore, due to their recalcitrant nature, microplastics interact with other contaminants present in the

environment, such as trace metals. Still, little is known about the mechanisms driving these interactions and the consequences that this co-pollution might pose to the biota and ecosystem function, and as such this study, therefore, focuses on assessing and determining the pollutants (i.e., microplastics and trace metals) present in (relatively) pristine mountain areas, where the presence of microplastics is mainly derived from atmospheric deposition. First, this study aims to determine whether there is any correlation between the presence of microplastics and trace metals in different compartments of the river ecosystem. Second, we also aim to determine the likely effects of these two groups of pollutants on river biota. Because our study sites are subjected to different impacts (current and historical), we also aim to determine the possible sources of microplastics and trace metals. For this purpose, we analyse microplastics and trace metals present in river trout (*Salmo trutta*) and other ecosystem compartments (e.g., biofilm) in the Pyrenees. The mercury concentrations up to 500+ $\mu\text{g kg}^{-1}$ w.w. measured in trouts, occasionally even exceeding EU guidelines of maximum threshold values, are clearly associated with a bioaccumulation phenomenon as demonstrated by the correlation between size, $\delta^{15}\text{N}$ signatures and mercury concentrations. On the contrary, microplastic concentrations identified by Nile Red fluorescence and FTIR, as well as other trace metals, are not associated with bioaccumulation mechanisms but rather with specific characteristics of the habitats. Finally, we will assess the risk this co-pollution may pose for other components of the trophic chain, including humans.

3.10.P-We096 Microplastics in Seafood From the Coastal Areas of Semarang, Java, Indonesia

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Indonesia is considered to be the world's second largest contributor to the amount of plastic waste in our oceans. However, there is little data on the abundance of microplastics in the Indonesian marine environment. This is particularly true for microplastics in seafood, which is widely consumed in Indonesia. The objective of this study was to determine the contamination levels and properties of microplastics in commercial seafood from the coastal region of Semarang, a densely populated city on the Indonesian island of Java. Three types of seafood were collected: milkfish (*Chanos chanos*), blood cockle (*Anadara granosa*), and green mussel (*Perna viridis*). Microplastics were found in almost all seafood samples (97 -100%). The highest abundance was observed in green mussels (70.7 ± 48.0 particles/individual), followed by blood cockles (18.3 ± 7.3 particles/individual) and milkfish (5.9 ± 4.3 particles/individual). The seafood samples were dominated by fragments, with the exception of milkfish, which was dominated by fiber-shaped particles. Beads and pellets were discovered as well. Microplastics in the size range of 50 - 100 μm were mainly found in bivalve samples, while particles in the size range of 100 - 1000 μm were mainly found in milkfish. Polymers of various types were discovered in the seafood samples. The composition of microplastics in milkfish was dominated by rubber, cellulose, styrene copolymers, and polyamide. In blood cockles, the top three plastic polymers were cellulose, polyamide, and polyethylene, while in green mussels, rubber, cellulose, and styrene copolymers were the top three. In parallel with the sampling of seafood, the occurrence of microplastics in sediments and water was examined and it was found that the occurrence of microplastics was higher in the vicinity of residential areas. These results confirm that microplastics contaminate our environment and our daily diet, so further research into the potential health risks from ingestion of microplastics is urgently needed.

3.10.P-We097 Temporal Variability of Microplastic and Anthropogenic Particles Uptake by Four Commercial Species From Arcachon Bay (North East Atlantic , France)

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Microplastics (MP) overrun every marine compartments of the world. Their small size ($< 5\text{mm}$) make them easily ingestible by a wide range of organisms. In this study, MP uptake at several seasons was described in four commercial marine species from the Arcachon Bay: wild Pacific oysters (*Crassostrea gigas*), common sea bass (*Dicentrarchus labrax*), common sole (*Solea solea*) and sea spider crab (*Maja brachydactyla*). In total, 304 individuals were analysed in the framework of the whole study. After a chemical digestion of digestive tracts (fish and spider crab) or soft tissues (oyster), a visual sorting was made under a binocular to extract highly suspected MP-like items from 26 μm (oyster) or 50 μm (fish and spider crab) to 5 mm. Then, a chemical identification was performed by infrared spectroscopy. After visual sorting, particles were recovered in all species at each sampling time. Maximal concentration was found for sea bass in January (6.80 ± 9.19 item.ind⁻¹). Items were mainly cellulosic in sea basses and soles (at least 78%) while similar proportions between cellulosic and plastic items were found in spider crabs (around 36%). In all species, fiber-shaped items were overwhelming (at least 78%). Cellulosic fibers pollution in marine environments is likely to stem from abrasion of fabrics. Focusing on MP (i.e. cellulose excluded), concentrations tended to be more shlgly higer in spider crabs (from 0.53 ± 1.06 MP.ind⁻¹ to 1.40 ± 2.41 MP.ind⁻¹) compared to sole's MP uptake (from 0 MP.ind⁻¹ to 0.62 ± 0.46 MP.ind⁻¹). MP concentration for sea bass range from 0.27 ± 0.59 MP.ind⁻¹ to 0.33 ± 0.65 MP.ind⁻¹. Polyethylene (PE) and polyester (Pes) were mainly identified in fish species (at least 23% for Pes and 25% for PE) while polyamide was predominant in spider crab (28%). Ecological traits such as feeding behaviour or trophic level may explain these differences as already suggested in the literature. These preliminary results will be completed by wild oysters analysis and inferential statistics to check at quantitative (i.e. concentration) and qualitative (i.e shape, color and polymer type) temporal variability. Moreover, data on MP contamination of sea surface, water column and sediments will help in understanding these results and may give insights on the transfer pathways from abiotic to biotic compartments.

3.10.P-We098 Microplastics in Polychaeta and Sediments From Offshore and Coastal Areas in Norway

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The global concern about microplastics (MP) has grown considerably since their first detection in the marine environment and wildlife. Ocean sediments may constitute the ultimate sink for MPs, both for denser plastics such as nylon, polyethylene terephthalate (PET) and polyvinylchloride (PVC) and for low-density polyolefins (e.g. PE and PP) which may aggregate and increase density over time due to biofouling. Still, little is known about the concentrations and impacts of MPs in benthic ecosystems. A previous study has seen potential bioaccumulation of microplastics in tube-dwelling polychaeta when compared to MP concentrations in their surrounding sediments. These polychaetes burrow in sediments and embed their tubes with particles from their surroundings, thus potentially also MPs. These results indicated that the feeding behaviour and life cycle of polychaetes could have an important influence on the transport, distribution and food-chain dynamics of microplastics in sediments. The present study aims to investigate potential differences in MP levels and quantify MP in sediments and polychaeta from offshore and coastal areas in Norway. The study also aims to investigate potential differences in MP levels in tube-dwelling and deposit feeding polychaeta without tubes. A total of 9 sediments samples (0-1 cm) and 24 polychaeta samples, both tube-dwelling and deposit feeding, were collected from offshore and coastal areas in Norway between 2017 and 2020. The offshore samples were collected on the Norwegian Continental Shelf and in the Barents Sea, while inshore samples were collected at Ålfjord, Lindesnes, Nordgulfjord and Oslofjord. Offshore sediments were sampled at remote locations or adjacent to oil platforms, which constitutes a probable local source of microplastics other than aquaculture and shipping in these areas. The inshore sediments are most likely more affected by anthropogenic sources of microplastics such as emissions from waste-water, various industry, aquaculture and boat traffic. Analyses of MPs > 45µm in sediments and polychaeta were performed using density separation with ZnCl₂:CaCl₂ solution (ρ = 1.52 g/cm³), and chemical digestion using a twostep procedure with NaOH:thiourea:urea to dissolve cellulosic material and further chemical oxidation with NaOH and H₂O₂. Finally, the samples are analysed using µFT-IR which will be performed during December 2021. Funding was provided by the Norwegian Retailers' Environment Fund.

3.10.P-We099 The Effect of Ageing on the Behavior of Poly(Lactic Acid) Particles As a Potential Vector for the Transport of Metals in the Presence of Natural Organic Matter

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Plastic production presents an exponential growth over the years since their commercial development. Unfortunately, only a small portion of this plastic is recycled when discarded, while most of it is released directly into the natural environment where it can persist for several years until its complete degradation. To overcome this issue, there is a new trend on the development of bio-based polymers, which are eco-friendly and biodegradable, to replace conventional petroleum-based plastic products. The presence of microplastics (MPs, fragments < 5 mm in diameter) in the environment is of great concern due to their adsorption/desorption capacity toward chemical substances such as organic compounds and metals. Here we present a physicochemical study on the adsorption/desorption of metals to commercial poly(lactic acid) microspheres (PLA) that is considered a biodegradable plastic. The pristine and aged PLA were characterized through FTIR, Raman, SEM, and N₂ BET isotherms, as a function of the duration of different degradation treatments. We obtained kinetic and equilibrium information on the binding of metals by pristine and aged PLA in aqueous media considering the effect of variables such as pH, ionic strength, and the presence of natural organic matter. These results represent a preliminary study to assess the risks of using biodegradable plastic products and provide the key factors that should be considered concerning potential hazard environmental effects. *Acknowledgments* – The authors thank the support from the European Union's H2020 research and innovation programme under Marie Skłodowska-Curie grant agreement No 801586, and Grants PID2019-107033GB-C21 and PID2020-117910GB-C21 funded by MCIN/AEI/10.13039/501100011033. P.L. also acknowledges support from the Ministerio de Ciencia, Innovación y Universidades of Spain and University of Lleida (Beatriz Galindo Senior award number BG20/00104).

3.10.P-We100 Eco-Corona Formation on Plastic: Effect of Plastic Type and Aging

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Concerns about the adverse environmental impacts of nano- and microplastic are continuously increasing, yet the understanding of plastic fate in freshwater environments is still limited. Plastic chemistry, size, shape, density as well as the freshwater composition all influence plastic particle fate. Aging of plastics, either by photochemical weathering or by adsorption of (bio)macromolecules which leads to formation of an ecocorona, can further change the physicochemical properties of plastics. However, the impact(s) of photochemical weathering on the physicochemical properties of plastic, and how this impacts subsequent adsorption of (bio)macromolecules and eco-corona formation, is often neglected. Here we conducted systematic studies on adsorption of a selection of ubiquitous (bio)macromolecules (humic & fulvic acids) to a set of the most environmentally prevalent polymer types (polyethylene, polypropylene, polyethylene terephthalate & polystyrene) in their pristine and photochemically weathered forms. Using Quartz Crystal Microbalance with Dissipation (QCM-D) allowed for determination of changes in mass of adlayers on the sensor surface. We spin coated various polymers onto the QCM-D sensor surface followed by passing solutions of (bio)macromolecules dissolved in synthetic freshwater over the polymer coated quartz crystals to monitor adsorption over time, as measured by changes in the resonance frequency of the piezo crystal. The extent of ecocorona formation on pristine plastics was compared to plastic films which underwent photochemical weathering. Both pristine and UV aged plastic films were characterized before ecocorona formation by contact angle measurements (changes in polarity and charge), Fourier

Transfer Infrared spectroscopy (chemical changes) and Atomic Force Microscopy (changes in physical surface roughness). Photochemical weathering of the pristine polymer results in decreased adsorption of (bio)macromolecules in synthetic freshwater which is expected due to the increased surface polarity and more negatively charged surface. This information will help to understand the rate and extent of ecocorona formation for dissolved organic matter on different pristine and photochemically weathered plastics. Ultimately, this will allow us to produce more realistically aged materials to be used in further experiments on fate of plastics.

3.10.P-We101 Effects of Microplastic and Fine Particulate Organic Matter (FPOM) on Aquatic Invertebrates - Putting Microplastic Effects Into Perspective

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The growing demand for products containing plastics has led to an increasing release of plastic waste into the environment. Plastic residues enter the hydrological cycle and gradually break down into tiny particles (< 5 mm), so called microplastics (MP). Freshwater systems have been recognized as a major pathway carrying over 2 million metric tons of plastic into the world's oceans every year. These lotic ecosystems are receiving significant amounts of plastic litter either during surface run-off events or via sewage and domestic wastewater treatment plants. Besides a permanently growing awareness of potential effects posed by MP on aquatic biota, knowledge on effects towards leaf-shredding freshwater organisms and its impact on microbial litter decomposition, as a catalyst for ecosystem functioning, is still scarce. To assess the effects of MP on the physiological performance of shredders and on microbially mediated leaf litter decomposition, we performed three 28-day-bioassays using the freshwater isopod *Asellus aquaticus* and three particles, polystyrene (PS), polymethyl methacrylate (PMMA), and fine particulate organic matter (FPOM). As a natural particle, FPOM was chosen to put potential effects into perspective. *A. aquaticus* is considered as both a grazing and shredding macroinvertebrate that nourishes of aquatic hyphomycetes (AH) on the leaf's surface, as well as on the actual leaf itself. AH are crucial for the microbially mediated leaf decomposition and the palatability of the leaves to aquatic detritivores. Each experiment was split in two parts, each part with one control and five treatment groups (n=10) with final particle concentrations of 0.18, 1.8, 18, 180, 1800 particles per milliliter. During the first part, 5 individuals of *A. aquaticus* from a laboratory-owned culture and sets of 30 pre-weighed and pre-conditioned leaf discs were added to the microcosms to assess the effects on the physiological fitness of the isopods. To monitor the microbially mediated leaf litter decomposition, the second part with 10 additional replicates per treatment, was set up without any isopods but with an adjusted number of 18 leaf discs per replicate. This study represents an important linkage between the effects on organisms and the impact on the longitudinal nutrient transport within streams and rivers through the effects on the microbial community on the leaf's surface as a part of the detritivore system and as a food source for shredders. Preliminary results showed that particles did not impact the survival of *A. aquaticus* however, PS significantly decreased the feeding rate. To get a more comprehensive overview on the impact of different concentrations of MP, further analysis will be performed to evaluate (i) the effects on the physiological performance of *A. aquaticus*, (ii) the impact on their gut microbiome, as well as (iii) to assess further effects on the microbially mediated leaf litter decomposition and on fungi and bacteria density.

3.10.P-We102 Co-Exposure With an Invasive Seaweed Exudate Increases Toxicity of Polyamide Microplastics to the Marine Mussel *Mytilus galloprovincialis*

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Marine environments face ongoing multiple pressures. For instance, invasive species are considered a major threat to marine biodiversity. The red seaweed *Asparagopsis armata* is considered a highly invasive species, which is known to exude a myriad of secondary metabolites as a defence mechanism that may have detrimental effects on native organisms. Microplastic pollution is also an issue of growing concern, especially for marine biota. Polyamide microplastics (PA-MP) are a group of polymeric debris commonly detected in marine environments deriving from fisheries activities. A major risk associated with these ubiquitous pollutants is their bioavailability to marine organisms, potentially affecting filter-feeders such as the mussel *Mytilus galloprovincialis*. This species is abundant and widespread in the Iberian coast with a major ecological and commercial value, being largely produced and consumed by local populations. *M. galloprovincialis* not only is known to ingest and accumulate microplastics, it is also known to be negatively affected by *A. armata* exudate. However, joint exposure of these co-occurring stressors has not yet been addressed. Thus, the aim was to assess the potential impacts of PA-MP exposure (1 mg/L) in *M. galloprovincialis* alone and combined with *A. armata* exudate (2%). For this purpose, byssus production as well as biochemical responses associated with oxidative stress and damage (in the gills), energy metabolism and production (in the muscle) were measured. Byssus number declined in mussels exposed to PA-MP either in the presence or absence of the exudate, with a lower number of byssus / mussel being found in the combined treatment. Decreased investment on byssus production is in accordance with the changes observed at the sub-cellular level and high energetic requirements to deal with oxidative stress. Oxidative damage at the protein level reflected the depletion in antioxidant capacity under combined stress. Increased ETS (Electron Transport System) activity and consequent increment of aerobic energy production was observed in mussels exposed to PA-MP treatments. This was accompanied by a depletion of CEA (Cellular Energy Allocation) activity representing a decrease in the energy budget especially when PA-MP were combined with the exudate. Overall, results reveal more severe effects of PA-MP on

M. galloprovincialis in the presence of *A. armata* exudate, suggesting that combination of these stressors may pose a threat to the marine biota.

3.10.P-We103 The Adsorption on Microplastics Reduces Phototoxicity of Titanium Dioxide Nanoparticles

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Over the last decades, the ecotoxicological effects of microplastics and nanoparticles have been intensively studied. Researchers have focused on the individual effects of these pollutants, although they can occur simultaneously in the environment, as both are commonly used in cosmetic products. In this context, the aim of this study was to evaluate the interactions and ecotoxicity of polyethylene microplastics (MPs), used as abrasives in cosmetic products, and titanium dioxide nanoparticles (nTiO₂), commonly used as UV filters in sunscreens. First, the interactions between MPs and nTiO₂ were investigated and the results showed that nTiO₂ is rapidly adsorbed on MPs (72% in 9 hours), which is most likely explained by chemisorption. Scanning electron microscopy and element mapping showed a uniform distribution of nTiO₂ on the surface of MPs. The interaction between nTiO₂ and MPs was strong and limited the desorption of nTiO₂ into the surrounding media. In the second part of the study, the ecotoxicity of MPs, nTiO₂ and MPs loaded with nTiO₂ was investigated for *Daphnia magna* (largely following the OECD 202 guideline) under visible light and ambient UV light. MPs, nTiO₂ nor MPs loaded with nTiO₂ did not affect *Daphnia* when the experiment was performed under visible light, but the presence of UV light (24 h) resulted in increased inhibition in all treatments. Under UV light, 100% of *Daphnia* were immobilized at all concentrations of nTiO₂, while the inhibition caused by the combination of UV light and MPs loaded with the same amount of nTiO₂ was approximately 30% lower and more comparable to the results obtained for MPs only. The plausible explanation is that nTiO₂ produced reactive oxygen species (ROS) under UV light throughout the entire volume of the test vessel, while nTiO₂ adsorbed on MPs produced ROS only locally near the surface of MPs limiting their contact with *Daphnia* that were located at the bottom of the test vessel. These results suggest that MPs and nTiO₂ may interact with each other and influence their ecotoxicological profile.

3.10.P-We104 Microplastics As Vector for Pollutants in Soils - Influence of Polyethylene Microplastics on the Bioaccumulation of Ivermectin in *Eisenia fetida*

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The ubiquitous occurrence of microplastics is increasingly recognized as a global concern. Microplastics, which can consist of many different polymers, enter the food chain. However, research has so far focused primarily on aquatic ecosystems. Knowledge regarding the impact of microplastics on soil organisms such as earthworms (lumbricids) is still limited. Especially concerning soil fertility and the food chain, it is controversially discussed whether microplastics serve as a vector for pollutants and thus influence the accumulation of certain lipophilic compounds. In this study the very hydrophobic antiparasitic agent ivermectin, which is applied extensively in veterinary medicine worldwide and enters the soil via animal excretions, is used. To examine the influence of low-density polyethylene (LDPE) microplastics on the accumulation of ivermectin in earthworms, a bioaccumulation experiment with *Eisenia fetida* was carried out according to OECD Guideline 317. For this purpose, the earthworms were kept in artificial soil for a period of 21 days. The soil was spiked with ivermectin at a concentration of 0.5 mg kg⁻¹ and 0.3% (w/w) LDPE (particle size 200-500 µm) was added. Soil and earthworm samples were collected at specific intervals and analyzed via high performance liquid chromatography using fluorescence detection (HPLC-FLD) after derivatization. Furthermore, the gut content of *Eisenia fetida* was also collected and analyzed. Bioaccumulation factors (BAFs) were then calculated and the uptake kinetics were modeled. The results were compared with a control (without LDPE). Finally, to obtain visual evidence for microplastic ingestion by *Eisenia fetida* a method was developed to examine the gut contents using Nile red staining with a fluorescence microscope. The first preliminary results of this study will be the subject of the current poster.

3.10.P-We105 Is the Ingestion of Microplastics Related to Feeding Habits and Trophic Levels in Fish Species? A Novel Approach Based on Stable Isotopes Analysis

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The Mediterranean Sea is a crucial biodiversity hotspot and has been defined as one of the most affected areas by plastic pollution worldwide. This study aims to assess the impact of microplastics on 253 individuals from 8 fish species (*B. boops*, *E. encrasicolus*, *M. merluccius*, *M. poutassou*, *M. barbatus*, *S. pilchardus*, *S. scombrus* e *T. trachurus*) sampled in the Ligurian Sea (Northwestern Mediterranean Sea). The focus of the study was to investigate how factors such as habitat, trophic level and feeding habits may influence or be related to the microplastics ingestion. To define the trophic niches and trophic levels of each specimen, analysis of the stable isotopes of ¹³C and ¹⁵N in the muscle of all sampled specimens were carried out. In all eight species analyzed, ingestion of plastics was confirmed and a total of 139 plastic particles were isolated. The species with the highest microplastic occurrence (%) was *B. boops* where 37% of individuals had ingested microplastics, followed by *S. pilchardus* (35%). *M. merluccius* was the least impacted with 8.3% of individuals with microplastics in their GI tracts. The microplastics detected and characterized were mainly polyethylene (67%), followed by polypropylene (20%) and polystyrene (9%). No significant correlation between microplastic occurrence and trophic level, isotopic niche width (SEAc), trophic diversity (CD) and nitrogen

range (NR) was observed. A significant positive correlation between microplastics occurrence and increasing diversity of basal sources used (Carbon range) was found. Planktivorous species such as sardine and bogue show the highest occurrence of MPs and the greater diversity of basal resources used. Moreover, a significant negative correlation between the number of ingested items per individual and the trophic level was observed. This relationship could be explained by the different feeding habits of the 8 analyzed species; at lower trophic levels we have planktivorous/pelagic species (anchovies, sardines, bogue) while the benthic predator species have higher trophic levels (i.e. red mullet, hake). This also agrees with the wider trophic niche (SEAc) exploited by benthic species. Overall, an influence of trophic niche descriptors on the occurrence of microplastics ingestion was not observed, but it seems that at the increase of the diversity of basal carbon sources used, the occurrence of plastic ingestion raises significantly.

3.10.P-We106 The Combined Presence of Microplastics and Hg Affects Hg-Resistant Fungal-Community Activities?

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Estuaries are heavily impacted by anthropogenic contamination, serving as reservoirs of various pollutants such as heavy metals (e.g., mercury (Hg) and microplastics (MP, $\varnothing < 5\text{mm}$)). These pollutants can affect water quality and cause toxic effects in aquatic organisms. Mercury, a trace heavy metal, is characterized as neurotoxic, persistent, and bio-accumulative in the food chain. In systems contaminated by Hg, microorganisms are responsible for reducing Hg, contributing to its natural detoxification. However, the effects of the presence of MP in this process are still unknown. The growing concern of micro-plastics is not only the chemical additives in their constitution that can be released, but also their ability to act as vectors of other contaminants, namely Hg, and microbial community biofilm, the so-called “plastisphere”. Therefore, the following questions arise A) Does the presence of microplastics and Hg affect Hg-resistant fungal-community and activities? To answer this above-mentioned question, the fungal community was isolated from a Hg-contaminated area of the Tagus estuary (Barreiro), and the most resistant strains were selected to assess the effect of the presence of polystyrene MP (PS) (40 mg/L) on fungi activity in a Hg-containing medium. After 1, 5 days, and 10 days of incubation of Hg-resistant strains with PS MPs and Hg (1ppm), the following factors were analyzed: A) Hg detoxification capacity and B) acute toxicity associated with the leached (Microtox bioassay). Furthermore, the isolates were identified through the amplification ITS. The results showed that: (i) fungal strains isolated from the Tagus Estuary exhibit high Hg-resistance (MIC range 37.5 – 50.0 ppm), (ii) Hg concentration in the medium were reduced via fungal activity (65-93% of the control), (iV) however, the presence of MPs increased the toxicity associated to the leached. Thus, we can conclude that MPs interfere with fungal activity in a Hg-contaminated aquatic system by increasing the toxicity associated.

3.10.P-We107 Individual- to Population-Level Effects of Smoked and Non-Smoked Cigarette Filters in the Deposit Feeding Polychaete *Capitella teleta*

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Cigarette filters (CFs) are the most dominant item in litter clean up surveys. In the environment CFs deteriorate into smaller fibres and leach an array of chemicals trapped in the filter. Thus, CFs may pose an environmental risk due to the release of plastic fibres and associated chemicals. It is recognised that CFs may affect both pelagic and benthic organisms, but little information is available on their long-term effects at higher levels of biological organization. The aim of the present study was to assess the effects of smoked CFs (SCFs) and non-smoked CFs (NCFs) on individual life-history traits and extrapolate these to possible effects at higher biological organization (i.e., populations). The benthic polychaete *Capitella teleta* was exposed to sediment-spiked granule of SCFs and NCFs at an environmentally realistic concentration (0.1 mg fibre g⁻¹ dw sed) and a 100-fold higher (10 mg fibre g⁻¹ dw sed) concentration. The experiment was divided in a juvenile and an adult exposure phase. In the juvenile exposure 10 larvae (5 days old) was exposed and monitored weekly for survival and time to sexual maturity. Once worms reached sexual maturity a male and a female were paired (adult experiment) and monitored every other day. The endpoints included somatic growth as well as survival, time to first reproduction, time between reproductive events and reproductive output. Overall, juvenile survival decreased with time in all treatments. After three weeks of exposure juvenile survival was significantly lower for worms exposed to SCF at high concentration compared with the control. Similarly, the presence of SCFs in the sediment affected the number of surviving worms that reached sexual maturity within three weeks of exposure when comparing with control. High concentration of SCFs significantly affected number of worms reaching sexual maturity, whereas a borderline significant effect was detected at low concentration. Overall, none of the tested endpoints in the current experiment was significantly affected by the presence of NCFs in the sediment. The initial results of the current experiment indicate that chemicals associated with the smoked filters are causing the effects rather than the cellulose acetate fibers from the filters in itself. Ultimately, measured life-history traits are to be incorporated in a simple demographic model to project changes in population growth rate in order to assess potential effects of exposure to CFs on population level.

3.10.P-We108 The Effect of Leachates From a Ultraviolet Weathered Bio-Based Composite on Marine Diatom Growth

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Polymers made from a natural-sourced feedstock, like polylactic acid (PLA), known as bio-based polymers, are seen as more

sustainable alternatives to petroleum-based polymers, with lower carbon and environmental footprint. However, concerns are emerging on whether these bio-based polymers have a lower ecological impact than petroleum-based polymers. In the marine environment, plastic additives can be released, i.e. can leach, from plastic to the surrounding seawater and organism. Studies have demonstrated the negative effects of leachates from several petroleum-based polymers on marine organisms, however, to date, few studies have assessed the potential effect of leachates from bio-based polymers. As part of the Interreg 2 Seas Mers Zeeën project SeaBioComp (seabiocomp.eu), we aim to assess the effect of leachates from a newly developed bio-based composite (i.e. bio-based polymers integrated with synthetic or natural fibres) on the growth of a marine diatom; and explore whether the effect is enhanced or diminished by UV radiation. To do so, we exposed the marine diatom *Phaeodactylum tricorutum* to a dilution series of leachates from pristine and weathered self-reinforced (SR-) PLA, following the ISO 10253:2016 protocol. To obtain weathered plastic, SR-PLA sheets flakes were subjected UV radiation, simulating up to 18 months of natural solar exposure. Our results indicate that neither leachates from pristine SR-PLA nor from UV-weathered SR-PLA had an effect on the growth of *P. tricorutum*, and UV radiation had no effect on the toxicity of SR-PLA leachates. In addition, we determined the EC₅₀ (i.e. half maximal effective concentration) to algal growth of two potential leached compounds, which were 1.86 ± 0.06 mg/L for dodecan-1-ol and 341 ± 30 mg/L for di-lactide. We anticipate that our results will contribute to assessing the ecological impacts of bio-based polymers and composites which may be more sustainable alternatives to petroleum-based polymers.

3.10.P-We109 Mapping of Occurrence of Microplastics in the Sediments of a Stormwater Pond

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In the modern world, dependency on plastic polymers for extensive commercial, industrial, medicinal and municipal applications is inevitable. Stormwater, wastewater and combined sewer overflows are commonly considered significant potential sources of Microplastics (MPs), discharging to waterbodies in urban areas. In this study we investigate the spatial distribution of MPs in the sediments of an inland stormwater pond located in the city of Aarhus, Denmark. To examine if MPs are evenly distributed in terms of location, size and polymer composition and the driving factors on these spatial patterns, thirteen sediment samples were collected. Briefly, samples underwent physical and chemical treatments including preoxidation, Sodium Dodecyl Sulfate (SDS) treatment, enzymatic treatment and density separation. Samples were sieved through a 500 µm mesh. Microplastics below 500 µm were identified and quantified by microscopy coupled to a Fourier Transform Infrared microscope (µFTIR with Focal Plane Array). Considering both number and mass, the average MP concentration was $44'396.86$ item kg⁻¹ and $11,839.98$ µg Kg⁻¹. There was no systematic variation of neither number nor mass concentration in the pond. Although samples were taken from different locations, small-sized low dense Polypropylene particles were the most abundant MP type in the majority of the sampled sites. Therefore, it can be concluded that deposition of MPs in the pond's sediments did not follow any pattern corresponding to their size and type. In the other word, MP distribution in the sediments was not size and type (density) selective. The fact that lighter-than-water-polymers were the most abundant types, and that there was no systematic relationship between position in the pond, concentration, and composition of MPs indicates that the transport mechanism of MPs from the water to the sediments was not simple sedimentation, but that the deposition was governed by some other mechanism. This study contributes to gain knowledge of the processes and mechanism which drives transport of mp in shallow water systems.

3.10.P-We323 Microplastics in the Rhizosphere: Consequences on Root Exudation and Microbial Communities

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Important agricultural crops are able to establish symbiosis with the microbial community in agricultural soils, forming mutually beneficial exchanges of photosynthates for nutrients that are otherwise inaccessible to the plant. These root-soil-microbial interactions are honed over millions of years of evolution and predicated on naturally occurring soil properties which can be altered by microplastics. Plastic particles from biosolids added to soil as a nutrient source are potentially ecotoxic and may interfere with normal root functioning through several mechanisms, including deleteriously affect soil properties, blocking signaling molecules, absorb soil organic matter important for roots and soil microbes, and may release toxins that exert a selective pressure against the microbial community. To investigate biosolid microplastic effects on the root-soil-microbial interactions of three crops (corn, soybean, alfalfa) ranging in their association with root-symbionts (fungal partnership only, fungal partnership with N fixing bacteria, alternative fungal partnership and N fixing bacteria) crops will be grown in agricultural soils amended with artificially created microplastics that reflect reported biosolid microplastic properties (i.e. size, quantity, type, surface morphology), yet are free of other potentially toxic substances that are variably found in biosolids and could be confounding variables. The presence of microplastics in the rhizosphere is envisioned to disrupt root-soil-microbial interactions will can be quantified via rot exudate analysis of rhizosphere soil and soil microbial community functional profiling. As roots alter the physicochemical properties of the adjacent soil through root exudation to increase nutrient availability, stabilize of toxic metals, and protect against pathogens using anti-microbials [5] it is plausible microplastics may induce similar stresses and thus alter the magnitude and composition of low-weight molecules transferring from roots into the soil. Exudation can suppress microbial metabolism pathways and thereby alter their utilization pattern of soil carbon [6] quantifiable by the MicroResp method which monitors the evolution of carbon dioxide from microbial respiration in soil amended with various C sources.

3.10 Microplastics in the environment: Behaviour, transport, fate, risks, and alternatives to conventional plastics (Part V)

3.10.T-22 Do Microplastics and Pharmaceuticals Interactively Affect Mussels? An Insight Into Uptake, Tissue Distribution and Metabolomic Profiles

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Mussels are suitable model organisms in ecotoxicology studies of pharmaceutically active substances (PhACs). Antidepressants and lipid regulators constitute good examples of relatively bioaccumulative PhAC classes ($\log K_{ow} > 3$), which co-occur in the aquatic environment with other contaminants of emerging concern, such as microplastics (MPs). MPs can adsorb pollutants on their surface, impacting their bioavailability and associated effects. In this study, we aimed to evaluate the interactive effects of polyethylene MPs and selected PhACs through a comprehensive assessment of PhAC bioconcentration, tissue distribution and depuration. In addition, a non-targeted analysis approach (LC-HRMS) is implemented to explore alterations in metabolic signatures and metabolism of PhACs by mussels. A bioassay was designed using Mediterranean mussels (*Mytilus galloprovincialis*) exposed to polyethylene MPs 4-6 μm (PE), the antidepressant citalopram (CP), the lipid regulator bezafibrate (BZ), and the corresponding PE-PhAC mixtures, besides a control without contaminants (CT). Mussels were sampled twice during exposure (10 and 21 days) and after a depuration period of seven days, extracting hemolymph, digestive gland and whole tissue samples. Elevated concentrations of citalopram were found in mussels at 21 d (mean: 729 ng/g dw), which surpassed bezafibrate by three orders of magnitude (2 ng/g dw). Accordingly, moderate bioconcentration factors (BCFs) were reported for the antidepressant, in the range of a previous study, and regardless of PE-MP presence (1279 – 1535 L/kg). Partition from seawater to mussel hemolymph (P_{HW}) was also higher for citalopram than bezafibrate. In this context, PE-MPs seemed to delay the internalization of citalopram, with diminished whole body levels in co-exposed mussels after 10 days ($p < 0.05$). In addition, an enhanced excretion of the parent drug in MP-unexposed mussels, as observed in depuration seawater, may indicate interactive effects on drug biotransformation and excretion. Future work will provide insights into affected molecular pathways, as well as possible impacts on the metabolism of PhACs.

3.10.T-23 The Leaching of Phthalates From Polyvinyl Chloride Microplastics Into Aqueous Environments

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Excellent material properties and raising demand have led to an increasing production of plastics from 50 Mt in 1976 to 348 Mt in 2017. Polyvinyl chloride (PVC), the third most used polymer in the European Union PVC contains the highest amounts of additives amongst the most widely used polymers, especially phthalic acid esters (phthalates). Additives can transfer to the surrounding environment within the lifespan of the plastics. Leaching of phthalates from plastics changes the mechanical properties of the plastics and results in embrittlement and fragmentation of larger plastic pieces to microplastics. Phthalates like bis(2-ethylhexyl) phthalate (DEHP) pose a risk to the environment due to their endocrine disrupting effect. For the environmental risk assessment and modelling of the fluxes of plastics and its associated additives in-depth knowledge of leaching kinetics of additives from (micro-) plastics into aqueous environments is required. The release of additives and non-additives from microplastics is controlled by internal and external diffusion, i.e., intraparticle diffusion (IPD) and aqueous boundary layer diffusion (ABLD), respectively. The diffusion process of some organic contaminants like polycyclic aromatic hydrocarbons (PAHs) and polybrominated hydrocarbons (PBCs) from microplastics to aqueous systems has been identified. Yet data on leaching kinetics of phthalates to the aqueous environment are not available. In this work, we used an infinite sink method using activated carbon powder to investigate the leaching of phthalates from PVC microplastics into aqueous environments. Batch leaching experiments were conducted over a time span of 120 days with six different microplastics containing ~23% to ~38% of bis(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DINP) or dioctyl terephthalate (DOTP). Thereby, time dependent leaching curves for each phthalate could be obtained. These leaching curves were evaluated using models for IPD and ABLD. The aqueous boundary layer diffusion model could well match the experimental data and could be identified as the governing diffusion process. Elucidating the governing diffusion process allowed us to calculate specific desorption times (e.g., desorption half-lives) of phthalates in aqueous environments. Our findings contribute to better understand the fate and behaviour of microplastics and phthalates in the aqueous environment.

3.10.T-24 Microplastics and Microalgae As Vectors for Chlorpyrifos and Mercury Toxicity in the Marine Copepod *Acartia tonsa*

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Plastic pollution has become a world-wide concern that can potentially threaten the stability of aquatic communities. While it remains unclear whether microplastics (MPs) introduced in the marine systems may or may not be toxic on their own, these particles have the capacity to interact with ubiquitous environmental contaminants, potentially acting as a vector of toxicants to marine organisms. The purpose of this study was to test this hypothesis using the calanoid copepod *Acartia tonsa* and two toxicants, a metal: mercury (Hg) and a hydrophobic organic compound: the organophosphate insecticide chlorpyrifos (CPF), and as model polyethylene MPs: high-density polyethylene (MPP-635XF) and oxidized high density polyethylene (Aquatex 325). The individual effects of Hg, and their combined effects with polyethylene MPs, on the survival, fecundity, feeding and egg viability of *A. tonsa*, were determined. Furthermore, this study aimed to compare the role of MPs and natural organic particles (microalgae, MA, *Rhodomonas lens*) as vectors of Hg and CPF toxicity. *A. tonsa* individuals were exposed to oxidized polyethylene MPs (0.25, 0.5, 1, 2 and 4 mg/L), to Hg (0.01, 0.1, 1, 10 $\mu\text{g/L}$) and to the combination of Hg and MPs (0.5-8 $\mu\text{g/L}$ of Hg + 0.25-4 mg/L of oxidized MPs). No significant effects were observed after exposure to oxidized MPs, but significant toxicity was found after

exposure to Hg on survival (LC50 = 3.55 µg/L), egg production (EC50 = 2.31 µg/L), hatching (EC50 = 3.88 µg/L) and recruitment rate (EC50 = 2.64 µg/L). However, the presence of MPs decreased the toxicity of Hg obtaining LC50/EC50 values of 6.67, 5.44, 4.03 and 2.49 µg/L, for survival, egg production, hatching and recruitment rate, respectively. On the other hand, the obtained results showed similar toxicity of Hg and CPF in copepods exposed to loaded MPs and MA, indicating that MP are vectors of CPF and Hg to the same extent than MA.

3.10.T-25 Biofouling Causes Tolerance of *D. Magna* to Microplastic Stress

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Ingestion of microplastic (MP) cause detrimental effects on organisms by diluting the nutritional value of food, but can also be beneficial if nutritious biofilms are present on the MP surface. This renders the question: is ingestion of MP harmful or beneficial and can the net effect of the two mechanisms be quantified? In this work, we quantified these harmful and beneficial effects on *Daphnia magna* (*D. magna*), using dose-response tests with clean and biofouled MP respectively, and determined the trade-off between these counteracting effects. For this end, polyethylene fragments were aged in natural water samples with different composition and organic load, namely river downstream and upstream a wastewater treatment plant (WWTP), and the effluent and influent from the same plant. The results of toxicity evaluation indicated that the environmental biofouling caused tolerance to MP exposure on *D. Magna* immobilization. The usual dose-response curve was observed for pristine MP. Pristine MP also showed higher immobilization of *D. magna* than biofouled particles. We found that the negative effects of MP can be compensated by the positive effects of biofouling layers as a food source. The survival rate of *D. magna* could be attributed to the amount of the resulting biofilm (carbon content) formed on MP. The highest survival rate was observed for MP biofouled in WWTP influent for 21 days, which had the higher content of biofilm. We successfully developed a theoretical modelling approach which simulates population growth by incorporating dose-effect relationships for ingestion of MP and ingestion of food, with part of the food being biofilm from biofouled MP. The model was applied for both pristine and biofouled MP. The applicability and accuracy of the model was tested by comparing model predicted profiles with experimental data ($R^2=0.868-0.991$). We also plotted isoclines for zero population growth, separating MP concentration and food concentration combinations that support *D. Magna* population growth from the combinations that jeopardize population growth due to ingestion of MP. These results contradict previous studies were only pristine MP were used and demonstrate that the ruling paradigm of unambiguously adverse MP effects is not ecologically justifiable.

3.10 Microplastics in the environment: Behaviour, transport, fate, risks, and alternatives to conventional plastics (Part VI)

3.10.T-26 Influence of Microplastics and Freshwater Snow Heteroaggregation on Particle Settling Rates in Freshwater

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To date, many studies have focused on the detection and quantification of microplastics (MPs) in natural waters, but only a few have been conducted to systematically understand the processes which drive MPs fate and transport in freshwater ecosystems. This includes hetero-aggregation with suspended natural colloids and small biota, and how these interactions affect distribution and transport in freshwater (e.g. settling dynamics). Freshwater snow (FWS), a mixture of algae and natural particles, is responsible for most of the flux of organic matter from the water surface to the sediment and can potentially act as a vector for MPs through the water column. Here we systematically analysed the settling rates of particles of different densities and morphologies in a plexiglass column, illuminated by a laser to track particles with a stereoscopic camera system. Image sequences were acquired and analysed with a tracking software to determine settling rates of the particles in three test systems: 1) MPs of various composition, size and morphology, 2) FWS and 3) MPs-FWS heteroaggregates. For each experimental set, our setup allowed the tracking of up to 2×10^4 particles, which ensured statistical convergence of the results. FWS was created by mixing freshwater algae (*Asterionella*, *Mycrocistis*, *Kirchneriella*) on a roller table to simulate the formation of natural flocks over time. Our goal was to assess if the settling rates changed when plastic and FWS were aggregated together, compared to their settling dynamics individually. While data analysis is still ongoing, we hypothesize that MPs size, shape and extent of incorporation into FWS will be important variables in the settling dynamics of the materials. Through this work, we demonstrate the utility of our experimental approach (camera setup, laser and the tracking software) for the analysis of particle settling dynamics – which has been validated with spherical particles of different sizes and densities according to Stokes law. Collectively, we provide further insights on the settling rates of MPs which can be used as input values for future fate models in freshwater systems, as well as an indication as to how the presence of MPs can impact the biogeochemical cycles through altering FWS settling in freshwater.

3.10.T-27 Science to Solutions: From Characterising to Tackling Microplastic Pollution in the Galapagos Islands and the Wider Eastern Pacific

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Plastic leakage from the Eastern Pacific rim countries of Ecuador and Peru is captured by the South Pacific Subtropical Gyre, transporting it towards the open ocean, where it pollutes oceanic islands such as Galápagos, famous for its unique biodiversity and a global conservation priority. Here we report on our collaborative programme working to first quantify the widespread plastic contamination of the Galapagos marine ecosystem and then to develop both local and regional monitoring and management plans

with regional stakeholders with the aim of reducing plastic input and wildlife impacts for this iconic archipelago. A two-year field sampling campaign focussing on the island of San Cristobal was undertaken, sampling the sea surface, benthic sediments, beaches and representative invertebrate biota for microplastics in combination with beach macroplastic surveys. Our data reveals widespread microplastic contamination of San Cristobal of both benthic sediments (6.7–86.7 particles·kg⁻¹) and surface seawater (0.04–0.89 particles·m⁻³), with elevated concentrations in the harbour suggesting some local input. Microplastics were present in all seven marine invertebrate species examined, found in 52% of individuals (n = 123). Highest beach plastic accumulation was on the eastern coast indicating major input of plastic waste to Galapagos from the Humboldt Current. A systematic priority scoring analysis identified the most vulnerable vertebrates as pinnipeds, seabirds, turtles, and sharks due to risk of entanglement and ingestion. Finally, we developed citizen science projects to provide ongoing time series data and recommend a monitoring and management framework for marine plastic pollution in remote oceanic islands, supporting the strategic efforts of the Galapagos National Park Directorate.

3.10.T-28 The Shapes of Microplastics

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Microplastics (MP) are ubiquitous and potentially pose risks to organisms. The environmental fate of MP is not only associated with its density and size, but also the shapes. Common shapes for MP are ‘*fiber*’, ‘*fragment*’, ‘*film*’, etc.. Some of them require determination of the thickness (the third dimension), which is feasible for MP above 300 µm using optical microscopy. The majority of MP are smaller sized, their identification relies on hyperspectral imaging, but this only analyses particle’s 2D projection. This means that some of the current shape categories are unsuitable for the most environmentally abundant MP. This study aims to derive a set of 2D shape categories for MP identified by hyperspectral imaging. The proposal is based on practical experiences of experts who work closely with MP analysis using this technique. The shapes were then used as references to evaluate the shape of 11,402 environmental MP (identified by FPA-µFTIR imaging), aiming to investigate patterns of MP shapes in the environment. We asked 5 experts to give input on 1) what are the often-seen pixelated MP shapes? 2) which 2D geometries can represent the pixelated shapes? Then we proposed 9 categories: *fiber*, *rod*, *ellipse*, *ovoid*, *sphere*, *quadrilateral*, *triangle*, *free-form*, and *unidentifiable*. These categories were used to evaluate the shape of the 11,042 MP extracted from indoor air, wastewater influent, effluent, sludge, stormwater, sediments, and marine water. The evaluation was conducted individually by the experts, and the degree of distinguishability of the shapes was also assessed. The most abundant shape was *ellipse* (19.1%), and the least was *fiber* (2.3%). But *fiber* was the most distinguishable shape, while *ellipse* was the least. Freshwater MP were differentiated from that of air and marine water regarding particle shape, and the differentiation on particle size showed a clear phase-based pattern: MP from solid-phase samples had the largest size, indoor air MP had the smallest. Hyperspectral imaged MP need suitable 2D geometries for shape categorization. These shapes must be relevant to the natural MP shapes, but also feasible for manual assessment. The proposed shapes are not meant to serve as the universal MP shapes categories. Nevertheless, they give a clear picture of the distribution of MP shapes in the environment. We finally suggest that MP shape should be considered in environmental mapping studies to enhance the applicability of such data for environmental impact assessment.

3.10.T-29 Environmental Safety of Second and Third Generation Bioplastics in the Context of the Circular Economy

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Biodegradable plastics designed using organic materials other than crude oil are often suggested as sustainable solutions for tackling end-of-life plastic waste, but little is known of their ecotoxicity to aquatic species. Here, we investigated the ecotoxicity of four second (2G) and third generation (3G) plastics (i.e. those using organic by-products, residuals, or waste feedstock (2G), in our case pork, fish or shellfish waste, or algae (3G) feedstock), toward the freshwater zooplankton *Daphnia magna*. In acute toxicity tests (48h), survival was impacted only at high concentrations (g/L range), similar in range to salinity-induced toxicity. All tested bioplastics decreased exposure media pH suggesting the potential to induce acidification stress. Algae-based bioplastics induced hormetic responses under chronic exposure (21d), enhancing most biological traits compared to controls (reproduction rate, body length, width, apical spine, protein concentration) from 0.06 to 0.5 g/L. Phenol-oxidase activity, indicative of immune function, was also enhanced at the lowest concentrations (62 and 125 mg/L). These suggested health benefits were hypothesised to be due to assimilation of carbon derived from the seaweed-based bioplastic as food. Also, the seaweed-bioplastics showed a complete compost disintegration and a readily biodegradation in freshwater, suggesting a low environmental persistence. In conclusion, the tested bioplastic showed promising environmental safety, classified as ‘not harmful’ under current global regulations; further studies are needed to better understand their longer term ecological and multi-generational effects.

3.11 One Health next generation wastewater management and reuse: the role of non-target and retrospective analysis, bioassays, and wastewater surveillance (Part I)

3.11.T-01 Integrating Panel Studies, Survey Data and Wastewater Based Epidemiology for Illicit Drug Use Monitoring; the Amsterdam CASE

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Drug consumption estimates are of relevance because of public health effects and associated criminal activities. Here we illustrate the combined use of Wastewater Based Epidemiology (WBE) of drug residues to estimate drug *use* and panel studies and survey data to reveal information from and on *users*. We present the triangulation of ten years of monitoring Amsterdam's cocaine, amphetamine, methamphetamine and MDMA *use* and *user* data. WBE revealed significantly higher consumption during weekends of MDMA (+41%) and cocaine (+26%). This observation corresponded to qualitative information from panel studies for MDMA, cocaine use setting. Furthermore a significant increase in *per capita* consumption of all stimulants was observed between 2011 and 2019, this was not reflected in the panel and survey data. However, the observed decrease in use during the COVID-19 pandemic 2020-2021 did corresponded to the obtained information from users. These different types of studies that reveal different information enable to connect observed trends in total drug consumption to behavior of users and the social context in which the use takes place.

3.11.T-02 The Role of Wastewater-Based Epidemiology As a Complementary Information Source During the COVID-19 Pandemic in Belgium

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Wastewater-based epidemiology (WBE) is a complementary approach to monitor substance use in the general population. The aims of this study were to: (i) evaluate intra- and inter-year temporal changes in stimulant and alcohol use during the first wave of the COVID-19 pandemic (14th of March 2020 till September 2020); and (ii) measure the effect of the COVID-19 restrictions on substance use. Daily 24-h composite influent wastewater samples were collected from the beginning of September 2019 until the end of September 2020 in four wastewater treatment plants corresponding to four Belgian cities (i.e. Antwerp, Boom, Brussels and Leuven). Human metabolic excretion products of amphetamine, cocaine (i.e. benzoylecgonine), ethanol (i.e. ethyl sulphate), 3,4-methylenedioxymethamphetamine, and methamphetamine were measured in these samples using a validated method based on liquid chromatography coupled to tandem mass spectrometry. Measured concentrations (ng/L) of human biomarkers were converted to per capita mass loads (mg/day/1000 inhabitants) by multiplying with the daily flow rate and dividing by the catchment population. The temporal fluctuation in population size was accounted for through the inclusion of mobile phone data as a dynamic population proxy. Day-to-day temporal changes during the COVID-19 pandemic were assessed, and the effects of the COVID-19 interventions on alcohol consumption were investigated. In most of the investigated locations, stimulant use remained stable or even increased in 2020 compared to 2019. Consumption of stimulants did not change, or was even higher, in the full lockdown period compared to the exit strategy and relaxations period. Similar to stimulant use, the consumption of alcohol did not significantly decrease during the lockdown period compared to the pre-lockdown period (starting from September 2019 till March 2020). However, in contrast to illicit drugs, alcohol use increased during the relaxation period after re-opening of the catering industry and increasing the allowed number of contacts per household. The present study shows the potential of WBE to rapidly assess the impact of COVID-19 interventions on alcohol and illicit drug consumption in Belgium. This study also indicates that WBE could be employed as a complementary data source to fill in current knowledge gaps linked to lifestyle behavior during the COVID-19 pandemic.

3.11.T-03 Determination of Phthalate Monoesters in Wastewater As an Indication of Human Exposures

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Phthalates are synthetic organic chemicals commonly used as plasticisers in PVC and additives in personal care products. Over 213 million kilograms of phthalates are produced each year with end uses including food packaging, flooring, paints, tubing and medical devices. (EPA 2006). Due to the ubiquitous nature of phthalates in the environment they are considered emerging pollutants. As a result, their toxicity has been thoroughly investigated leading to their classification as endocrine disruptors; having effects on male reproductive health, children's neurodevelopment, obesity and cancer. High molecular weight phthalates have been investigated less frequently but may have the same effects on health. The majority of studies on phthalates focus on environmental presence of phthalate di-esters or biological presence of phthalate mono-esters. The ability to monitor both is beneficial to examining the life-cycle of these compounds and how pervasively they persist in biological systems. Exposures to phthalate di-esters were estimated from the mono-ester metabolite concentrations in influent using flow rates, population served and correction factors for phthalate metabolism. Levels were found to be between 0.32 to 277.25 µg/inhabitant/day. This paper demonstrates the development of an analytical method for the determination of phthalate metabolites in urban wastewater to demonstrate likely human exposure levels in the environment.

3.11.T-04 Occurrence and Fate of Pharmaceuticals and Personal Care Products in Crops Irrigated With Reclaimed Water

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Wastewater reuse for agricultural irrigation has increased worldwide in the recent years due to water scarcity and climate change. However, wastewater treatment plants (WWTPs) do not have the capacity to completely remove emerging contaminants, such as pharmaceuticals and personal care products (PPCPs), and thus they are still present in these irrigation waters. Therefore, the possibility that these compounds can be uptaken by the crops intended for human consumption is real. The occurrence of 56 PPCPs in three types of crops, namely tomatoes, lettuces, and carrots, as well as in soil and irrigation water, was evaluated. WWTPs secondary effluent water and reclaimed water produced by the effluent infiltration through reactive barriers of a managed aquifer recharge (rbMAR) were tested for irrigation. Besides water quality, the effects of soil composition and irrigation system

on the PPCPs crops uptake were also investigated. The extraction of the target compounds from the different crops and soils was carried out by a QuEChERS-based (Quick, Easy, Cheap, Effective, Rugged and Safe) method, while the water analyses were performed by on-line solid-phase extraction coupled to high performance liquid chromatography and tandem-mass spectrometry (on-line SPE-HPLC-MS/MS). The results showed that the best combination of variables to lower the plants and fruits uptake of PPCPs was to irrigate with the reclaimed water, in soil with high content in clay. The irrigation system appeared to have low influence on the PPCPs uptake. A significant and differential bioaccumulation was observed with a total load of the selected PPCPs up to 1248.53 ng/g dw, 1691.76 ng/g dw and 7786.63 ng/g dw in the lettuces, tomatoes and carrots, respectively. These findings have significant implications for the reuse of wastewater in agriculture intended for human consumption. Contamination control in source and more efficient wastewater treatments are needed for a safe use of reclaimed water in agriculture in compliance with current water reuse regulation Acknowledgements: The authors are grateful to the Spanish Ministry of Science and Innovation (Project ROUSSEAU CTM2017-89767-C3-1-R and Project CEX2018-000794-S), Project RESTORA (ACA 210/18/00040) and Consorci d'Aigües Costa Brava Girona (CACBGi) for the unlimited access to the facility and treated and reclaimed water supply for irrigation, and to BEKOLut®, for the provision of the QuEChERS kits.

3.11.T-05 Soil Self-Cleaning Capacity: Removal of Persistent and Mobile Organic Compounds During Sub-Surface Irrigation With Sewage Effluent

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While significant attention has been paid to the more arid regions of the world, temperate climates also suffer from seasonal droughts. The pressure on the availability of freshwater will continue to increase due to climate change, which will enhance prolonged dry periods. Hence, it is crucial that alternative freshwater resources such as sewage treatment plant (STP) effluent are explored. When using sub-surface irrigation for agriculture as method of supply, the quality of STP effluent may improve due to the soil passage and related biodegradation processes. Nonetheless, the extent to which the dispersion of the mixture of contaminants of emerging concern (CoECs) is diminished in sub-surface irrigation systems is not yet fully understood. Consequently, the aim of this study is to identify and quantify CoECs from a real-scale cropland that is directly irrigated with STP effluent using sub-surface irrigation. We also aim to elucidate the fate of CoECs during soil passage and relate this to CoECs physicochemical properties. The selected area of study is the only Dutch cropland intentionally and directly irrigated with municipal effluent. In total 133 water samples taken from the rhizosphere and groundwater up to 12 meters were analyzed with a maXis 4G quadrupole time-of-flight high-resolution mass spectrometer equipped with an electrospray ionisation source (ESI-Q-TOF) to achieve MS detection. This study is complemented by more than a hundred targets that represent a variety of chemical structures and uses. The logD (pH=7.4) ranged from -2.77 (acesulfame) to 5.69 (flufenoxuron). The persistency (half-life) diverges from 0.4 days (Propanil) to 1194 days (2,6-Dichlorobenzamide/BAM). To correct for dilution by rainwater and groundwater, concentrations in the rhizosphere-and groundwater samples were corrected based on the effluent tracer saccharin. The targets were separated into four groups representing the four combination of low and high persistency (P) and mobility (M). Our results show that the fate of CoECs is highly dependent on their physicochemical properties and external factors of the SSI system. After the extremely dry year of 2018 the sub-surface irrigation system needs more time to recover, which can be correlated to the low amount of precipitation in the winter of 2018-2019 and resulting low dilution.

3.11 One Health next generation wastewater management and reuse: the role of non-target and retrospective analysis, bioassays, and wastewater surveillance (Part II)

3.11.T-06 Operational Application of Invertebrate Behaviour Videotracking for Online and Real-Time Wastewater Surveillance

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As availability of water resources decreases, the apparition of new contaminants of concern (CEC) identified in water networks continues to increase. Current wastewater monitoring in treatment plants relies on interval based grab sampling measurements for a select few chemicals or for punctual effluent toxicity assessment using a restricted choice of bio-assays. As this strategy is known to omit at large portion of micro-pollutants and contaminants of emerging concern (CECs), innovative approaches to wastewater management are essential to both avoid potentially detrimental impact of largely unknown CEC discharge in aquatic ecosystems and improve efficiency of urban water re-use. To better characterise micro-pollutant fate in wastewater treatment plants, we proposed a bio-innovative approach based on the video-tracking of locomotor behaviour of bio-indicator invertebrates (the crustacean *Gammarus*, the gastropod *Radix*, and the annelid *Erpobdella*), in an on-line wastewater multi-species monitoring device: ToxMate. For testing, certain CECs were selected according to French research and ground reports for both laboratory and WWTP testing (as the ToxMate apparatus is already present in French WWTPs). Our results illustrate the specific sensitivity of the bio-activity signal to the micro-pollutant discharge, as well as reporting results and feedback from real-case long-term monitoring sites which uncover moments of micropollutant linked water quality degradation. Finally, undergoing laboratory research on the definition of multi-species behavioural fingerprints to discriminate causes for CEC discharge is presented. The

success of this initial testing immediately showed the potential of such bio-innovation to better encompass contaminant discharge without sensitivity to environmental confounding factors. Continuous surveillance identifies critical moments that could be missed with current grab sampling techniques. These moments may help adapt wastewater management strategy in real-time. Thus, the potential is not only limited to the improvement of aquatic environmental quality through wastewater management, but could also provide concrete indicators of the suitability of urban water re-use.

3.11.T-07 Combined Chemical and Effect-Based Analysis for a Robust and Efficient Assessment of (Advanced) Wastewater Treatment

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Anthropogenic pollutants discharged into the aquatic environment via wastewater can cause negative effects on aquatic ecosystems despite very low concentrations. Therefore, there is a demand for advanced wastewater treatment technologies such as ozonation and/or the use of activated carbon but as well efficient methods to evaluate the removal of contaminants of emerging concern. Especially for the regulatory wastewater monitoring and operators of wastewater treatment plants, there is a need to monitor the success of the advanced wastewater treatment, not least because this is associated with significant investments and higher operational costs. In addition to the chemical analysis, effect-based methods are highly relevant to investigate the overall toxicity of treated wastewater, because they cover all substances contributing to an observed effect. Besides classic bioassays such as the Yeast Estrogen Screen or the Ames-Test to detect mutagenic effects a variety of methods was recently developed to combine chromatographic separation of compounds by thin-layer chromatography (TLC) with an effect-based detection directly on the surface of the thin-layer plate. To develop a robust and efficient approach to assess the success of (advanced) wastewater treatment by a combination of complementary chemical and effect-based parameters a systematic sampling of wastewater was performed along with the wastewater treatment processes including advanced treatment technologies. The samples were characterized using classic bulk parameters such as DOC, SAK, TNb and enriched by solid-phase extraction. The extracts were directed to a comprehensive chemical target monitoring and an effect-based assessment including mutagenic, genotoxic, hormonal and cytotoxic effects. Furthermore, a battery of planar bioassays - performed in a direct combination with thin-layer chromatography - was applied. The findings demonstrate that effect-based in-vitro methods are valuable complementary tools to chemical analysis since they detect existing, unwanted effects rather than compounds and thus directly indicate the quality of the treated wastewater. Especially bioassays in combination with thin-layer chromatography are robust and sufficiently sensitive to detect for example trace amounts of estrogenic compounds such as EE2 in the low pg/l-range.

3.11.T-08 Non-Target Screening of Pharmaceuticals Transformation Products in Urban Wastewater and Evaluation of Their Fate in Constructed Wetlands. a Retrospective Analysis

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The path of the contaminant from its discharge in the environment to the complete degradation to inorganic salts, water and carbon dioxide is rather long and complex. Although the state of knowledge on this subject is still insufficient, it has been demonstrated that some transformation products (TPs) may be as harmful as their parent compounds, in addition, they may be much more harmful in an environmental situation, where they occur in mixtures. In this study, two sampling techniques, passive and grab sampling, were compared for qualitative analysis of pharmaceutical TPs in urban WW effluents. Results demonstrated better suitability of passive sampling mainly due to the better quality of MS² spectrum. The non-target screening revealed the occurrence of 13 TPs in secondary treated effluents (i.e., tramadol N-oxide, N,O-didesmethyltramadol, amisulpride N-oxide, O-desmethylvenlafaxine N-oxide, O-desmethylvenlafaxine, N-desmethylvenlafaxine, venlafaxine N-oxide, gabapentin lactam, O-desmethyltramadol, clarithromycin N-oxide, hydroxycarithromycin, hydroxycarbazepine, and carbamazepine-10,11-epoxide). Retrospective analysis of the previously acquired data by high-resolution mass spectrometry was used to reveal the fate of these compounds during the treatment in constructed wetlands. Among identified compounds, N-oxides and carbamazepine-10,11-epoxide appeared to be the most recalcitrant, alongside amisulpride, gabapentin, and carbamazepine. The results obtained offer insight into the fate of pharmaceutical TPs and may serve as a guideline for the prioritization of contaminants TPs based on their persistence during the treatment and assist in the design of treatment systems.

3.11.T-09 tbd

3.11.T-10 Evaluation of Innovative/Alternative Septic Systems for the Removal of Contaminants of Emerging Concern

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Groundwater is susceptible to elevated nitrogen concentration and co-pollutants from a variety of sources, including onsite wastewater treatment systems. For example, conventional onsite septic systems can release high levels of both nutrients (e.g., nitrogen and phosphorus) and contaminants of emerging concern (CECs) to groundwater and, in turn, into nearby receiving surface waters. In some areas, centralized wastewater treatment is not practically possible due to cost, lack of political will, or

physical limitations; as such, septic systems should no longer be considered a temporary measure in advance of sewers but rather a permanent part of residential wastewater treatment infrastructure. Some coastal communities have been evaluating the removal of conventional septic systems and the installation of innovative/alternative (I/A) septic systems to reduce nutrient loads. Communities can select from various proprietary and non-proprietary I/A systems, each with its specific benefits and limitations. Enhanced I/A systems that include a lignocellulosic carbon source have been demonstrated to efficiently remove nitrogen from effluent along with some CECs. The removal of CECs during I/A treatment may be a co-benefit of installing these systems for residential wastewater treatment. Conversely, if some critical CECs are not removed (or only minimally removed) by the I/A treatment, communities may choose to opt for sewers instead of installing I/A septic systems. This project examines the removal from effluent of over 280 chemical and microbial CECs by conventional and I/A septic systems. Two conventional treatments were sampled: 1) a standard pipe-in-stone trench with 1.3 m of vadose zone, and 2) a drip disposal system where the septic tank wastewater disposal is in the active bio-zone (root zone) underneath the turf with percolate collected at a 0.6-m depth. Effluent was collected from 4 points in 3 different I/A systems: 1) a saturated wood layer system; 2) a denitrifying system with an aeration chamber and denitrification chamber; and a second denitrifying system with collection points after both the 3) nitrifying bed, and 4) wood chip bioreactor. This study will fill a critical information gap about the influence and/or mitigatory effect I/A systems have on ground and surface water quality as a diffuse source of CEC pollution. In addition, it will provide important contributions to the growing body of literature on the performance of I/A septic systems.

3.11 One Health next generation wastewater management and reuse: the role of non-target and retrospective analysis, bioassays, and wastewater surveillance (Virtual Only)

3.11.V-01 Evaluation of Comprehensive Quality Control Pipeline for Wastewater Surveillance of SARS-CoV-2

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Wastewater-based epidemiology (WBE) has been widely applied to track outbreaks of coronavirus disease 2019 (COVID-19). Viral load in wastewater is a promising community indicator of outbreaking magnitude. However, there are no standardized protocols for quantifying viral load in wastewater, especially for population normalization. Wastewater is complex and dynamic, which can impact the efficiency of viral enrichment, RNA extraction, and RT-qPCR. Additionally, community population is dynamics due to major events, which should be considered for long-term longitudinal surveillance. Here, a comprehensive quality control pipeline was proposed and evaluated to quantify the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA and key Variants of Concern (VOCs) in wastewater at Saskatoon, Canada. Internal and external spiking controls were applied to estimate recovery ratio and extraction efficiency. Wastewater parameters (daily inflow rate and ammonia) and population indicators (pepper mild mottle virus, acesulfame K and creatinine) were used to normalize viral load. Normalization methods were evaluated against the daily numbers of new cases of COVID-19 of the sewershed. Viral load was positively correlated with daily new cases reported in the sewershed. WBS had a lead time of approximately 7-day, which indicated surges in the number of cases of COVID-19 and revealed the major circulating VOCs with a and d driving the third and fourth wave, respectively. Adjustment with recovery ratio and extraction efficiency improved the correlation between viral load and daily, new cases. Acesulfame K performed better than pepper mild mottle virus, creatinine, and ammonia for population normalization. Normalization to acesulfame K improved the trend of viral load of the second wave during the Christmas and New Year holidays. Hence, the whole process controls to characterize recovery ratios and extraction efficiencies, and population normalization with Acesulfame K is essential for precise WBE programs supporting decision-making in public health.

3.11.V-02 Integrated Approach on Irrigation Management Using Non-Conventional WATER Resources Through Multidisciplinary Strategies for Risk Reductions

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Agriculture faces the challenge to produce more food to feed a growing population. The world population is expected to grow by 2.3 billion people by 2050, which will require extending the land equipped for irrigation by some 32 million hectares to guarantee raising overall food production by some 70%, since the per capita food consumption is also increasing. Under these circumstances, water withdrawals for irrigation to cope with the increasing food demands should grow by 11% for 2050. This intensifying water scarcity scenario represents a threat to the long-term role of irrigated agriculture in global food security, which cannot meet current or future demands for irrigation by relying solely on conventional water sources. New solutions are required to maintain or enhance sustainable agricultural production, including new or alternative water resources and water conservation strategies. In this context, an interesting technological approach is to increase the use of nonconventional water resources to guarantee long-term food security and socioeconomic stability. Although reclaimed water is commonly and successfully used in many countries, water reuse faces numerous barriers. Therefore, for the preservation of profitable intensive agriculture that respects the environment, are needed innovative agricultural projects incorporating state of the art technologies in water reuse with multidisciplinary studies. Sustainability of agriculture irrigated with low quality water will require a comprehensive approach to soil, water, and crop management consisting of site- and situation-specific preventive measures and management strategies. The objective of this work is to show a multidisciplinary approach on water reuse projects in agriculture through an integrated methodology, generating novel strategies based on the three new concepts on water reuse: “fit for purpose” (water quality should be adapted to the type of crops and irrigation techniques), “multiple barrier” (water quality for specific uses should result from a stepwise approach, cascade treatment technologies should allow for sequential quality upgrade according to changing requirements) and “risk-based approach” (water quality should be evaluated through the definition and assessment of risk levels that are appropriate for the specific use).

3.11 One Health next generation wastewater management and reuse: the role of non-target and retrospective analysis, bioassays, and wastewater surveillance (Poster)

3.11.P-Tu153 Wastewater Surveillance Supports COVID-19 Clinical Findings Across Epidemic Waves in South Africa

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Wastewater surveillance of non-infectious SARS-CoV-2 RNA fragments can provide an early warning for detecting COVID-19 cases in the community, detect variants, assess trends and track hotspots. This may in turn provide a cost-effective, rapid and reliable source of information on the spread of SARS-CoV-2 in communities. More than 18 months into the pandemic, South Africa has experienced three district COVID-19 waves with over 85 000 COVID-19 related deaths. Late November 2021, an increase of cases has been noted marking the start of the imminent fourth wave. The aim of this study was to describe and compare the difference in SARS-CoV-2 RNA levels in wastewater between 2 regions in the Western Cape (South Africa) during the second COVID-19 wave which coincided with the December 2020 holiday period. Both the Breede Valley and Theewaterskloof municipalities have four wastewater treatment plants (WWTPs) each. Wastewater influent samples were collected weekly from each of the eight WWTPs over a 12-week period. The total RNA was extracted with Qiagen RNeasy® PowerSoil® Kits and the SARS-CoV-2 RNA copy numbers were determined using quantitative Real-Time Polymerase Chain Reaction Analysis (qRT-PCR). The results showed that the SARS-CoV-2 viral loads from both catchments/municipalities followed the same trend as the clinical cases reported for the Western Cape province during the festive season. The two catchments are both located approximately 100 km inland from the coastal city of Cape Town, which is a popular holiday destination. The Breede Valley catchment is located on the national main road that stretches from Cape Town, across South Africa, through Bloemfontein and Johannesburg, and ends at the Zimbabwe border. Interestingly, higher SARS-CoV-2 RNA levels were quantified at the end of the second wave (4 January 2021) from Breede Valley compared to Theewaterskloof, potentially because this marks a peak travel period with people returning home. Wastewater surveillance of SARS-CoV-2 RNA proved to accurately reflect the clinical surveillance of COVID-19 infections in the Western Cape. In addition, the tool can be applied to retrospectively track the movements of people and advise on regulations for future outbreaks.

3.11.P-Tu154 Monitoring COVID-19 Through Wastewater-Based Epidemiology: Method Development, Disease Surveillance and Modelling in the South West UK

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Infectious disease outbreaks are a growing threat, as both the frequency and global impact of disease outbreaks are increasing. This is particularly exemplified by the COVID-19 pandemic, and urbanisation, population growth and rapid mass movement make such global disease outbreaks increasingly likely. Against such a background, improvements to disease outbreak monitoring are vital. This work proposes to address this challenge through the use of wastewater-based epidemiology (WBE). WBE is a field of research that treats wastewater as a fingerprint of a community's health. The quantification of nucleic acids in a single wastewater sample can help bring about an understanding of public health in a cheap and non-invasive way: indeed, it is estimated that the health of 27% of the global population could be monitored globally by analysing samples from just 105,600 wastewater treatment plants (WWTPs). To make best use of WBE, methodologies for the quantification of analytical targets must be fully characterised. To this end a study was carried out in the South West of the UK to develop a methodology for sample preparation and analysis of wastewater from 4 different WWTPs for the routine quantification of SARS-CoV-2 RNA, each with different catchment populations and industrial contribution proportions. Long-term surveillance of wastewater from these sites was carried out, enabling comparison with public health case data and the construction of a mathematical model. The impacts of sample composition, storage, pasteurisation and clarification upon SARS-CoV-2 RNA quantification were all assessed, as were the various efficacies of different concentration and extraction methodologies. Additionally, the precision and range of the methodology were assessed and inherent variabilities of the method were characterised, and the impact of sampling frequency upon disease outbreak modelling was also assessed. The findings from this study can be applied to the future development of a wastewater-based early-warning system for disease outbreak detection and/or surveillance. This work shows that, when fully characterised and understood, WBE can be a powerful tool for public health monitoring. In the hands of public health officials, WBE promises to enable timely and well informed decisions for the surveillance and control of disease outbreaks.

3.11.P-Tu155 Wastewater-Based Epidemiology in Valencia City (Spain): 8 Years of Drugs of Abuse Monitoring

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Wastewater treatment plants (WWTPs) can provide the ideal matrices to monitor quickly and efficiently spatial and temporal differences in the consumption or exposure to chemical substances or pathogens in a population by measuring certain compounds. This technique is known as wastewater-based epidemiology (WBE). In addition, given the high stability of certain compounds, through the WWTPs' effluents they can reach the surface and underground waters, impacting negatively the ecosystem. Our study focused on the analysis of six drugs of abuse and metabolites in the entrance of three WWTPs located in Valencia city (Spain). The sampling was carried out by collecting 24-hour daily composite influent samples for seven consecutive days from 2014 to 2021. Amphetamine (AMP), benzoylcegonine (BECG), cocaine (COC), methamphetamine (MAMP), 3,4-

Methylenedioxymethamphetamine (MDMA) and 11-nor-9-carboxy-D9-tetrahydrocannabinol (THC-COOH) were extracted by solid phase extraction (SPE) and detected by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Drugs were found in all samples analysed. Mean concentration values measured were high for cocaine compounds, BECG (1939.39 + 115.92 ng L⁻¹), COC (875.18 + 64.75 ng L⁻¹), followed by the cannabinoid THC-COOH (346.85 + 34.56 ng L⁻¹), and amphetamine type stimulants AMP (75.53 + 5.78 ng L⁻¹), MAMP (10.08 + 0.70 ng L⁻¹), MDMA (44.91 + 3.21 ng L⁻¹). A general significant increase in concentrations was noted in the weekend's samples but different trends were observed between years. Similar concentrations were also observed between WWTPs. The results of this study provided updated information about the consumption of these substances in the city of Valencia. They also highlight the importance of annual monitoring of the elimination efficiency of these compounds in WWTPs in order to decrease their environmental impact on the different ecosystems. Acknowledgements This work has been supported by Grant RTI2018-097158-B-C31 funded by MCIN/AEI/10.13039/501100011033 and "ERDF A way of making Europe".

3.11.P-Tu156 Spread of Antibiotic Resistance and Pharmaceuticals in Blackwater Reuse: From Source to Recipient Environment

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Reuse of domestic wastewater is of growing global interest and is a key part of the transition to a circular economy. As source-separated wastewater fractions, blackwater is a concentrated solution of human excreta and flush water from households, and thus is beneficial to nutrient recovery. Blackwater reuse in agriculture offers a promising solution to reducing the dependence on chemical fertilisers. However, the potential environmental impacts due to blackwater reuse are not yet fully understood. The aim of this study was to evaluate the dissemination of antibiotic resistance and other pharmaceuticals in an agricultural field with blackwater reuse as biofertiliser. Soil and nearby surface water samples were collected before and after blackwater application at increasing time intervals (up to 35 days). Blackwater treated with urea, for hygienisation, and samples from the recipient environment were analysed for pharmaceuticals including antibiotics, as well as antibiotic resistance genes (ARGs). More than half (57%) of the target pharmaceuticals were quantified in the blackwater with a concentration range of 23-154 000 ng/L. Compared the urea-treated blackwater stored for one year with that for four year, there was a reduction in the concentration of several antibiotics by an order of magnitude, except for ofloxacin. The rest of the studied pharmaceuticals remained similar in their levels over time. Although their detection frequency was lower in the recipient environment, a few antibiotics were persistent in the nearby water streams over time, especially ofloxacin and enrofloxacin. Similar results were also observed for other pharmaceuticals, such as carbamazepine and ibuprofen. Enrofloxacin was the only antibiotic that could be quantified in the soil samples over time. Interestingly, an increase in the relative abundances of antibiotic resistance genes was observed for blackwater after four-year storage compared to one-year storage. In the soil recipient, there was a general decrease in the number of resistance genes detected over time. However, for the clinically relevant ARGs, the relative abundances in the soil increased over time. Their occurrence in soil remained even after one year of blackwater application. Overall, our results showed a potential spread of antibiotic resistance and other pharmaceuticals via blackwater reuse, and also the need for better treatment strategies to remove antibiotic residues and other pharmaceuticals in blackwater.

3.11.P-Tu157 Prevalence of Antibiotics and Antibiotic-Resistance Genes in Dutch Urban Wastewater - a Case Study

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The spread of antibiotic resistance genes (ARGs) is increasing globally and putting pressure on the long-term effectiveness of antibiotics. Antibiotics are critical pharmaceuticals widely used in human health care for controlling bacterial infections. ARGs and antibiotic residues are often emitted indirectly to the environment via wastewater treatment plants (WWTP). Therefore, WWTPs are a steady source of antibiotics and ARG pollution. Considering the substantial technological and economical hurdles of increasing the removal efficiency of these pollutants, it is of interest to evaluate upstream emissions within the wastewater collection system to help devise alternative reduction strategies. In the present study, sewage samples from the city of Nijmegen (The Netherlands) were collected between Sep'2019 and Sep'2020 at 10 locations representing a variety of urban settings, i.e. hospital, elderly home, student complex, city centre, industrial park, residential area, rainwater pit and WWTP. In total, 23 antibiotics, 5 ARGs and 1 mobile genetic element were quantified and analysed. Site-specific profiles of antibiotic selective pressure and ARG were generated. Preliminary results show that the aggregated selective potential of antibiotics in wastewater positively correlates with the prevalence of ARGs. Furthermore, the city hospital, student residence and residential area constitute the main contributors to antibiotic and ARG emission in the sewage catchment.

3.11.P-Tu158 Identification of Toxic Drivers in Water Using Effect-Directed Analysis

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Anthropogenic organic micropollutants in the aquatic environment have shown to cause toxic effects (Rosenmai *et al.*, 2018; Lundqvist *et al.*, 2019) and has been recognized as an emerging cause for concern. Surface water contains complex mixtures of tens of thousands of organic micropollutants (Schwarzenbach *et al.*, 2006), challenging complete chemical characterization. There

is therefore a lack of knowledge on the toxic potential of organic micropollutants in water. One approach to identify organic micropollutants that drive toxicity is effect-directed analysis (EDA) (Brack, 2003). In EDA, fractionation of the sample is combined with bioassays and high resolution mass spectrometry (HRMS) analysis. Bioassays utilize cell based *in vitro* tests, which enables detection of the total bioactivity of an extract (Niss *et al.*, 2018), also taking unknown compounds and possible mixture effects into consideration. When the bioassay is conducted after fractionation of a water sample, identification efforts can be directed towards compounds that show bioactivity, and therefore are potentially toxic. The EDA approach thus saves both time and resources, and holds great promise for research as well as environmental monitoring. Unknown, toxic organic micropollutants in drinking water are of special concern. Since many drinking water contaminants are unknown or not well investigated, they are not covered by regulations and pose a potential threat to human health (Nilsen *et al.*, 2019). Studies have shown that only 0.1 % of the bioactivity observed in environmental water samples could be attributed to known, analyzed chemicals, for specific toxicity pathways (Neale *et al.*, 2017; Escher *et al.*, 2013). The unexplained bioactivity could either be attributed to unknown compounds or to mixture effects. By continuous improvement of analytical methods (Menger *et al.*, 2020; Gago-Ferrero *et al.*, 2018) more compounds – as well as mixture effects – can be identified, leading to a safer environment. In this project, a methodology will be developed and utilized, aiming to expand current knowledge on toxic drivers in water and mixture effects of organic micropollutants in the aquatic environment. Special focus will be directed at stormwater, wastewater treatment plants and landfills – which are important origins of pollutants in drinking water source areas (Rehrl *et al.*, 2020; Golovko *et al.*, 2021).

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3.11.P-Tu159 The Relevance of the Fate of CECs in Sewers - Results From a Meta-Analysis on CEC Half-Lives in Wastewater and Sewers

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Many chemicals of emerging concern (CECs) enter the environment via different pathways and put pressure on the quality of our resources - particularly water. While large amounts of empirical data on exposure and effects of CECs are currently being gathered, the immense number and variety of CECs used in society and industry calls for a more efficient and systematic approach to tackle this issue. Estimating the emission of CECs to the environment based on production and usage data requires modelling the chain of processes that take place between the production and use of a CEC and its release into the environment. Within this modelling chain, the fate of CECs in sewer systems is one aspect that has received relatively little attention leading to scarce and fragmented information on in-sewer fate of CECs. Therefore, we compiled information on sewer systems, in-sewer fate processes and existing modelling efforts. We concluded that biotransformation likely plays an important role in the fate of CECs upon passage through the sewer system. However, to model biotransformation we currently rely heavily on empirical half-life data. Consequently, we conducted a meta-analysis and collected half-lives (DT50s) of CECs in wastewater from various experimental set-ups including OECD 314A, bioreactor tests, pilot and field studies. The resulting dataset contains 277 DT50s associated with 96 unique compounds. 120 DT50s are shorter than 12 hours, and thus relevant for urban sewer catchments. The majority of entries (>83%) were reported for bioreactor tests, while only few entries were reported for pilot and field studies (10% and 7% respectively). Using the created dataset, we explored the relationships between DT50s and compound properties as well as between DT50s and experimental settings. Hereto, we performed correlation tests, principal component analysis and hierarchical clustering. However, except for some weak, yet significant, correlations between aerobic bioreactor DT50s and wastewater temperature ($R = 0.29, p < 0.05$), DT50s and area-to-volume ratio of biofilm ($-0.25, p < 0.05$), and geomean DT50 and $\log K_d$ ($R = -0.46, p < 0.05$), no meaningful patterns were found. With this poster presentation, we would like to present our data, methods and (partially inconclusive) results to open up a discussion on data quality, methodology and potential alternative approaches regarding both, experimental data as well as data analysis.

3.11.P-Tu160 Phosphorous in Wastewater: Speciation and Recovery With Polymer Inclusion Membranes

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Phosphorus (P) is a natural element considered an essential nutrient for its importance in the growth of living organisms. P can be found in waters in different chemical species, inorganic or organic, and different phases (dissolved, colloidal and particulate). In the dissolved phase, orthophosphate and condensed phosphates are the predominant species. The presence of phosphate at high concentration cause eutrophication in surface water bodies, and consequently a severe reduction in water quality [1]. The influent of wastewater treatment plants may contain around 4-15 mg L⁻¹ total P, coming mainly from urban leachates and anaerobic digested sewage sludge. Run-off from agriculture and pasture, where phosphate fertilizers are used, can also contribute to P loadings. Therefore, to maintain water quality, the removal of P is an important priority. P determination is commonly carried out by spectrophotometric methods (molybdenum blue method, PO₄³⁻), ion chromatography (PO₄³⁻) or using atomic emission techniques (P total). The determination of P organic species or colloidal P requires the use of more specific techniques and expert personnel [2]. Moreover, the recovery of P from wastewater has a great interest from both the economical point of view and sustainability. In this work, we present polymer inclusion membranes (PIMs) for P speciation and recovery. PIMs are a type of functionalized membranes synthesized in the laboratory and composed by two main components: a polymer and a carrier [3]. The polymer constitutes the skeleton of the membrane and in this work we have used cellulose triacetate (CTA). The ionic liquid Aliquat 336 (trioctylmethylammonium chloride) acts as the carrier for the ion exchange of anionic P species. The PIM is placed between the source phase (wastewater containing P) and a receiving phase containing NaCl solution, where P species are preconcentrated for detection or recovery. We have evaluated the speciation capacity of the PIM system by measuring different

inorganic P species and organic soluble species like adenosine 5'-triphosphate (ATP) and adenosine 3:5'-cyclic monophosphate (AMP). P recovery has been achieved by using a PIM mounted in a cell (spiral-wound) where the two phases were recirculated. **Acknowledgements** This work has been supported by the Ministerio de Ciencia, Innovación y Universidades (MCIU) through project PID2019-107033GB-C22 / AEI / 10.13039/501100011033 **References** [1] Guignard et al. *Frontiers in Ecology and Evolution*, vol 5, 2017. [2] Worsfold et al. *Analytica Chimica Acta* 624 (2008) 37-58- [3] Almeida et al. *Journal of Membrane Science* 415–416 (2012) 9–23.

3.11.P-Tu161 Going Into Circles: A Combinatory Approach to Investigate the Risks of Micropollutants and Their Transformation Products in Water Reuse Systems

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To maintain water security, availability, and quality, alternative water sources (AWS) offer great potential to make future water systems more resilient. AWS include, for example, rainwater, greywater, surface water, cooling water, or treated wastewater. However, water reuse only offers a real possibility if the associated risks for human and environmental health are thoroughly understood. Concerns for water reuse may arise as AWS are potentially contaminated with micropollutants (MPs) and their transformation products (TPs). Especially wastewater contains a broad range of MPs and TPs that are insufficiently removed or generated during treatment processes. However, for water reuse, the current legislative risk assessment and existing guidelines are insufficient. A clear focus by these guidelines was put on microbial safety to lower the risks of water reuse. Moreover, the current risk assessment pays little attention to TPs. Additionally, the risks for most MPs and TPs are only insufficiently known and have yet to be critically examined and assessed. By a combination of high-resolution mass spectrometry (HRMS) and effect-based monitoring (EBM), it is aimed to evaluate the fate and toxicity of MPs and TPs in various water reuse schemes. Target, suspect, and non-target HRMS screening will facilitate a comprehensive investigation of the chemicals present in reused water. To determine which water reuse schemes pose environmental or human health risks, EBM can help to elucidate harmful water samples and account for chemical mixture effects. To assess the potential of water reuse, different AWS, and different reuse schemes, we will present a schematization of water reuse which allows the definition of fit-for-purpose quality parameters. The schematization is based on the AWS, the applied treatment techniques, either advanced or nature-based, the reuse purpose, and associated protection goals. We present different potential case studies in the Netherlands within this schematization. Potential case studies include, for example, intentional recharge of groundwater through the infiltration of surface water, advanced treatment of treated wastewater that is discharged into the IJsselmeer, an important source for drinking water production in the Netherlands, or reuse of water for irrigation of green infrastructure.

3.11.P-Tu162 A General Framework to Address the Impacts of Salinity Variations, Including Emission-Related Positive Effects

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Salinity is changing in aquatic systems due to anthropogenic activities and climate change. These impacts can be even more uncertain in transitional waters. Although there are studies on the effects of salinity variations on individual species, little is known about the effects on overall ecosystems. The few works that do address this topic have considered these impacts using ecotoxicity models. But these models state that an increase in the concentration of a pollutant generates an increase in the impacts. However, the impact of salinity is also linked to concentration decreases. Moreover, salt is not a pollutant, but an essential element, so a critical improvement of these methodologies is necessary. Impacts linked to chemical releases are measured using the characterization factors (CFs). They consider the principal cause-effect chains – thus, fate and effect factors. CFs addressing impacts on ecosystem quality have units of potentially disappeared fraction of species (PDF)·m³·time/kg. The Fate Factor is expressed in units of time, and the Effect Factor is expressed as PDF·m³/kg. Here, the classic models used to define the effect factor are expanded to include effects linked to a decrease in the concentration of the substance, acknowledging the specific features of salinity. The proposed approach is based on the premise that the species in an ecosystem have an optimal range of salinity for living at which no impact occurs (i.e., PDF = 0), and that detrimental effects will be observed if it varies below or above it. Then, negative effects will occur if salinity increases above the optimal range or decreases below it. Moreover, positive impacts will occur for increments in the salt concentration for environmental concentrations below the optimal, and vice versa. Then, after defining the optimal salt concentration range, there are two EFs: EF_{LOW} and EF_{HIGH}, both in PDF·m³/kg. This research work addresses for the first time the potential effects on the environment derived from a decrease in the concentration of essential substances, where the effects of an emission can also generate positive impacts. Moreover, it is expected that the framework can also be applied to model environmental impacts of other essential substances in life cycle assessment, such as metals and macronutrients. According to the obtained results, salinity cannot be modelled using classic ecotoxic models (which address the issue considering salt as a pollutant). Therefore, the present study opens a new pathway to model how essential substances are modeled in the environment.

3.11.P-Tu163 Site-Specific Organic Contaminants Characterization of a Reclaimed WATER Irrigation System Through a High Resolution Mass Spectrometry-Based Non-Target Screening

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The unbalanced use of water between territories due to an unsustainable agricultural system forces, in some regions, the irrigation of crops and/or aquifer recharge with reclaimed water. The European legislation in these cases demands an extra treatment of this water after conventional wastewater treatment prior to its use. Despite the treatment efforts, this irrigation system may lead to the accumulation of pollutants in soils and plants. In contrast to the current monitoring techniques that only cover a limited number of targeted organic contaminants, high-resolution mass spectrometry-based non-target screening (NTS) permits to characterize these waters on a more holistic way and, thus, identify the most relevant pollutants in each case based on their occurrence and ecotoxicity. The Baix Llobregat Agrarian Park is an agricultural area partially irrigated with reclaimed water. In this study, a prioritization of the most relevant site-specific organic contaminants was performed. For this purpose, samples were taken from the water regeneration plant and from different points of the irrigation channels, during summer and winter. To avoid the loss of compounds during pre-treatment, the samples were lyophilised, re-dissolved in ethyl acetate and methanol, centrifuged, concentrated under nitrogen, reconstituted in the initial chromatographic conditions and, finally, filtrated. Analyses were performed with liquid chromatography coupled to high-resolution mass spectrometry using a Q-Exactive Orbitrap (Thermo Scientific) system. Both data dependent and independent acquisition modes were used. Then, the post-processing of data was carried out with the NORMAN software *Digital Sample Freezing Platform* and a NTS workflow to identify features and categorize each identified compound into the different levels of confidence, using the results obtained then for identification of the site-specific priority contaminants and evaluation of the treatment plan performance. Acknowledges: This work was supported by the European Commission (Project MAGO, PRIMA number 2022), by the Spanish Ministry of Science and Innovation (Project CEX2018-000794-S), and by the Generalitat de Catalunya (2017 SGR 01404).

3.11.P-Tu164 Chemical and Biological Assessment of WATER Treatment Using Novel Led-Based UV Reactors

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In the present research we investigate the applicability of highly innovative and sustainable technology (UV LED system) for chemical-free and efficient treatment of drinking water and wastewater. The efficiency and performance are compared also with other conventional treatments. The study focused on the assessment of installations of UV LED systems at end users in urban and rural areas in Japan (disinfection of community drinking water supply systems) and in the Czech Republic (final cleaning steps of urban waste waters). The samples of water were collected (and if relevant pre-concentrated using Solid Phase Extraction), and then assessed using a series of chemical and biological assays prior- and after-treatment. The endpoints included routine water quality parameters (such as turbidity, conductivity, N and P levels etc.), microbiological quality (total microbial activity, *E. coli*, *Pseudomonas* sp. etc), analyses of toxic metals (by ICP-MS), polar contaminants such as residual pharmaceuticals (15 analytes measured by LC-MSMS), bioassay for total estrogenicity (reporter gene assay) and ecotoxicological assays with algae, *Daphnia* and others. Preliminary data indicate that UV LED systems installed in the field are durable for extended periods of time and are efficient in removing of both microorganisms and trace pollutants. The unique direct comparisons with other treatment technologies are available thanks to parallel installations of both novel and standard approaches. [Acknowledgement – Supported by the EIG CONCERT-Japan programme via the international project “Innovative UV-LED applications to drinking water and wastewater treatment systems for sustainable water management in future communities (InLEDapp)” and by the Czech Ministry of Industry and Trade project No. CZ.01.1.02/0.0/0.0/19_262/0020109]

3.11.P-Tu165 Investigation of the Toxic Effects of Constituents in Textile Wastewater From WET Processing Using Effect-Based Methods

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Wastewater from the textile industry, especially wet processing, is one of the main factors for surface water pollution, especially in the central producing countries such as India. In India, the assessment of textile wastewater is currently limited to analysing physicochemical parameters such as pH, biochemical oxygen demand (BOD) and chemical oxygen demand (COD). However, the effects of chemical mixtures resulting from the complexity of textile effluents cannot be detected in conventional toxicity assessments but can lead to adverse effects such as acute toxicity, mutagenicity, and endocrine activity in organisms. In addition, the textile wastewater from India often shows high salinity, which leads to salinization of freshwater. If untreated or incompletely treated wastewater enters the environment, it can have serious consequences for the aquatic organisms and the ecosystem. Therefore, further test systems are needed to properly assess the complex mixture of components contained in textile effluents. In this context, effect-based methods (EBMs) are useful to detect the mode of action of chemicals. For this reason, one of the “EfectroH2O” project's goals is to establish EBMs in the form of a test battery that meets the specific requirements for the comprehensive analysis of textile wastewater (e.g., high salinity and complex chemical mixture). Samples of the dyes and the composition and concentration of salts in the wastewater were obtained from the textile industry MS/Rohini, Erode in Tamil Nadu in India. The acute toxicity of known components, including the salt concentration recorded at the textile dyeing plant MS Rohini, was investigated using established model organisms of different trophic levels. Among others, the bioassay battery included the fish embryo acute toxicity test (FET DIN EN ISO 15088) with the established freshwater model organism *Danio rerio* to

determine the acute sublethal toxicity of the salts in mixtures and singly. Additionally, *Daphnia magna* were used in the acute immobilization test (DIN 38 412 EN / ISO 6341) in combination with the algae growth inhibition test (DIN EN ISO 8692:2012). The project aims to reduce the toxicological impact on the environment by improving industrial process water treatment and additionally reducing water consumption in water-scarce regions such as India.

3.11.P-Tu166 Effect-Based Methods As Tool to Assess the Toxicity of Textile Wastewater

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During wet processing in textile production, which includes the dyeing of textiles, thousands of chemicals are applied, some of which are highly toxic. Alone the used dyes contain several toxic and stable compounds. If these get into the water system, for example due to the discharge of untreated or inadequately treated wastewater into rivers, this has serious implications for human and environmental health, as can currently be observed in major producing countries such as India. However, the impacts of industrial wastewater on organisms and the environment are difficult to assess because of the complex composition and mixture of chemicals present. The interactions of chemicals can lead to the formation of degradation products, resulting in more toxic compounds than the parent compound. Chemical analysis cannot fully detect the complexity of substances, which making it difficult to identify the drivers of toxicity. In addition, the high salt concentration in textile wastewater can lead to salinization of rivers, which can have an additional negative impact on the inhabiting freshwater organisms. A realistic risk assessment requires the use of holistic and impact-oriented methods. Therefore, within the German- India project, 'efectroH₂O', the adaption of effect-based methods to address the complexity of textile wastewater are investigated. Within the project two main approaches were followed. The first approach aims at the challenge of identifying suitable bioassays that can be used specifically for highly saline industrial wastewater. For this purpose, the species sensitivity of different model organisms was determined by exposing them to a specific salt composition. The composition of the salts is based on the original data of a dye factory in India (Ms/Rohini, Tamil Nadu). Within the second approach, wastewater samples from this dye factory were taken and fractionated by solid phase extraction so that the organic pollutants could be investigated without the influence of the salts using standardized bioassays. The acute toxicity on different trophic levels (algae, daphnia, fish) and specific endpoints like mutagenic, neurotoxic, or endocrine potential are investigated. In addition to the ecotoxicological investigation, chemical analyses of the wastewater sample was performed. By combining effect-based methods and chemical analysis, toxic drivers in textile wastewater can be detected and regulated.

3.12 Organic micropollutants in urban waters - occurrence, transformation, and treatment (Part I)

3.12.T-01 Predicted No-Effect Concentration (PNEC) - Based Design for Removal of Pharmaceuticals in Wastewater Treatment Plant

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In Denmark there are no regulatory demands for municipal WWTPs to remove pharmaceuticals. However, the polluter (e.g., an industrial unit) carries the environmentally related responsibility. A local hospital in northern Zealand (Hillerød), which will be replaced by a big regional hospital in 2025, discharges wastewater to a nearby WWTP (HCR Syd). The WWTP discharges the cleaned effluent to an environmentally vulnerable freshwater system, leading to strict effluent requirements. Moreover, the dilution factor of the effluent in the receiving water body is nearly zero. As a result, the utility is negotiating to take over the responsibility and is discussing design and target values with the local authority to reach the concentrations of active pharmaceutical ingredients (API) in the effluent down to the values of predicted no-effect concentration (PNEC). Based on recommendations from "Clear Waters from Pharmaceuticals" (CWPharma) guideline for advanced API removal "GoA3.4: Optimization and control of advanced treatment" (2020), pilot processes in the WWTP were tested treating the effluent with ozonation and granular activated carbon (GAC). Three treatment variations were applied: i) different ozone dosages, ii) GAC filtration, and iii) ozonation followed by the GAC filtration. Over 50 PNEC-referenced medicine-related compounds were measured with an LC-MS/MS, compared with the baseline effluent concentrations and with the latest PNEC values. Though GAC is spontaneously able to achieve all targets, extended use resulted in exceedance of some target values (e.g., for clarithromycin, venlafaxine. Ozonation effectively reduced most of the compounds but did not reach certain target values (e.g., for bicalutamide, oxazepam) even with 1 mg-O₃/mg-DOC. Besides, it alone can lead to formation and discharge of unfavorable by-products. The combination of both technologies reached effluent concentrations complying to all target values. However, in this project we also argue that PNEC values for certain compounds are set, lacking reliable toxicity data and comprise huge uncertainties. Adoption of more precise national or case-specific API targets would assist technology assimilation and benefit the environment. However, the ozone GAC combination seems to achieve all required removal for the target compounds.

3.12.T-02 Sartans and Sartan Metabolisation in Wastewater Treatment

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Sartans such as Losartan, Valsartan, Irbesartan are angiotensin II receptor blockers (blood pressure regulators). As they have become quite popular, their concentrations in wastewater are considerable (10 ng/L-2 µg/L). In this contribution we describe for the first time removal and metabolisation in a full scale activated sludge wastewater treatment plant in comparison to metabolisation in a biofilm reactor. The situation in the activated sludge plant is somewhat complex as several metabolites also originate from human metabolism meaning they were present in the raw wastewater. However, removal of Losartan, Valsartan and Irbesartan in the CAS WWTP were around 90, 90, and 40% respectively. 14 transformation products were characterized by high resolution mass spectrometry and structural formulas are verified by true standards or suggested based on product ion spectra analysed with high resolution mass spectrometry from samples originating from experiments in the biofilm reactor. The respective metabolites are also found in the activated sludge treatment plant, especially Losartan acid, Losartan carboxylic acid and Losartan TP453. Other metabolites Losartan TP 335, and Irbesartan TP447 stem to a high extend from human metabolism and are rather removed 40 and 90%, respectively than formed in the CAS-WWTP.

3.12.T-03 Use of Wood Pellets and Cork in Biofilters for the Simultaneous Removal of Nitrates and Pesticides From Groundwater

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About 13 and 7% of groundwater in Europe exceeds, respectively, the permitted levels of nitrates (50 mg NO₃⁻ L⁻¹) or pesticides (0.1 µg L⁻¹). Rapid Sand Filtration can remove nitrates via denitrification when oxygen is limited. For this process, microorganisms require organic carbon as an electron donor, which can be added by using natural and synthetic biopolymers in the Rapid Sand Filtration packing-material. Thus, we assessed the usage of wood pellets and cork as packing materials under water-saturated and -unsaturated conditions for the attenuation of nitrates and pesticides from groundwater. Biofilters were fed with groundwater spiked with nitrate (200 mg L⁻¹) and pesticides (5 µg L⁻¹) at an HLR of 95 mm d⁻¹. Nitrate, pesticides (atrazine, bromacil, diuron, and mecoprop) and their transformation products were monitored for >400 days. Microbial composition of the biofilter material was also characterized. In cork biofilters, nitrate attenuation decreased with time, reaching almost no attenuation. This indicates that organic-carbon release ceased, hindering denitrification. In the saturated wood-pellet biofilter, denitrification increased over time, reaching 99% removal. This could be due to the continuous release of organic matter from wood pellets and to the adaptation of microbial communities. Regarding pesticides, >99% of mecoprop was removed in all configurations but transformation products were detected in all biofilters except for the unsaturated wood-pellet one. Diuron degradation was >75% in all biofilters and no transformation products were seen. Atrazine was better removed in wood-pellet biofilters (68-96%) than in cork biofilters (31-38%). However, both unsaturated biofilters (cork and wood pellet) produced dealkylated atrazine. Finally, less than 20% of bromacil was removed in all biofilters but the unsaturated cork biofilter, which achieved >60% attenuation. Still, there, we measured a high intensity of a transformation product corresponding to bromacil oxidation, which questioned the applicability this configuration to remove bromacil. Microbial characterization suggests that higher diversity is linked to material composition and thus better biofilter performance. Both wood-pellet biofilters had a high relative abundance of Basidiomycota, which comprise white-rot fungi, known degraders of chlorinated compounds. Therefore, the use of wood pellet as a biofiltration material is effective to treat nitrates and pesticides from groundwater.

3.12.T-04 Removal of Urban Stormwater Runoff Organic Contaminants by Cost-Effective Reactive Media

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Growing populations, urbanization sprawl, and climate change poses a challenge on traditional cities' drinking water supplies. Stormwater harvesting schemes could replenish over-drafted groundwater resources, augmenting urban water supplies. However, urban stormwater runoff carries a myriad of dissolved contaminants (e.g., organics, metals, nutrients), which impair receiving water bodies. Moreover, some organic contaminants of urban origin —particularly persistent, mobile, and toxic substances (PMTs), like pesticides, plasticizers, flame retardants, etc.— may not be adequately removed by conventional infiltration treatments (e.g., blue-green infrastructures). Therefore, it is of the utmost importance to fully understand their fate, transport, and effect, while designing novel or upgrading conventional treatment systems. Pyrogenic carbonaceous materials (e.g., biochar) can adsorb trace organic and metal contaminants. Some manganese oxide-coated sands oxidize electron-rich organic contaminants and adsorb metals during stormwater infiltration. Thus, reconfigured green infrastructure systems with engineered media could provide means of contaminant removal by sorption and redox processes. To enhance the treatment performance of conventional media, herein we propose sustainable, low-cost and low-energy reactive geomedia (biochar and synthesized manganese oxide-coated sand). We have conducted preliminary laboratory-scale batch experiments to investigate their removal capacities. Our results showed that biochar displayed faster sorption kinetics and capacity compared to the other studied materials (manganese oxide-coated sand or conventional sand). Regarding our suite of PMT organic contaminants, the studied biochar exhibited non-linear pseudo first-order adsorption behavior, with sorption rate values (i.e., k_1) ranging from 0.860 to 3.983 h⁻¹, and showed adsorbed amounts (i.e., q_e) ranging from 0.015 to 0.021 mg PMT/g adsorbent. Most of the contaminant removal occurred within the first 24 h and equilibrium was attained within 48 h. Bisphenol A, which is not considered persistent, was the only compound removed (via oxidation process) by manganese oxide-coated sand due to its electron-donating moieties. Sand, commonly used in infiltration schemes, showed no reactivity, highlighting the need to study alternative materials to separate persistent, mobile, and toxic organic contaminants from stormwater runoff.

3.12 Organic micropollutants in urban waters - occurrence, transformation, and treatment (Part II)

3.12.T-06 Mass Balance of Diethyltoluamide (DEET) in the Aquatic Environment

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The biocide diethyltoluamide (DEET) is used worldwide as an insect repellent on human and animal skin. It is among the most frequently detected organic chemicals in wastewater, surface waters, and groundwater. The ubiquitous detection of DEET in Swiss waters, even during winter months, despite its apparent seasonal use as an insect repellent, has led to question the sources and fate of DEET in the environment and the validity of previous risk assessments for Switzerland. This study summarizes the occurrence, fate and emission dynamic of DEET in Swiss aquatic systems and attempts to clarify its sources to the Swiss aquatic environment. Environmental monitoring data are compared to newly obtained production and consumption data from the DEET Swiss supply chain, providing a first mass balance of DEET for Switzerland. The analysis of Swiss monitoring data suggests an annual Swiss consumption of 2 tons of DEET, which is consistent with the estimate of 2 to 12 tons derived from production data provided by manufacturers, distributors and formulators. These results indicate that, from a mass balance perspective, the widespread detection of DEET in Swiss waters can be corroborated by its consumption as an insect repellent alone. Based on these results, the established risk assessment methodology of the European Union and Switzerland under the Biocidal Products Regulation, the Emission Scenario Documents (ESD), were evaluated for DEET. It is shown that the ESD significantly overestimate the release and occurrence of DEET in Swiss surface waters and therefore provide a worst-case scenario that is consistent with the precautionary principle. In summary, this study provides a characterization of DEET occurrence in Swiss waters as well as its consumption to extrapolate a first indicative mass balance for Switzerland and compare this to established models for environmental risk assessment. This approach could be exemplary for other biocidal products whose emissions and occurrence are not well understood and which are characterized by higher ecotoxicity than DEET.

3.12.T-07 Wastewater of Formulating Pharmaceutical Industries As a Source for Peak Concentrations of Active Pharmaceutical Ingredients in Municipal Sewage Treatment PLANTS

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Pharmaceuticals in water bodies are of increasing concern due to their potential risk for aquatic organisms. A major source of emissions to surface waters are domestic wastewater treatment plants (WWTP) which also receive discharges from industries such as pharmaceutical production plants. Recent research indicates that these emissions significantly contribute to the load of active pharmaceutical ingredients in WWTP effluents and can cause short-term peak concentrations. However, knowledge on these emissions is scarce, especially for formulating pharmaceutical industries (FPIs). Therefore, this study aims to investigate a representative set of 8 WWTPs with inputs from FPIs throughout Switzerland. Since emissions from production sites are highly intermittent and enter the WWTPs as a pulse, sampling with high temporal resolution is crucial. To study the concentration dynamics, the MS2Field was deployed at the effluent of a WWTP. The MS2Field is a transportable trailer with a fully automated LC-HRMS platform, which can autonomously measure datasets with high temporal resolution. Every 20 minutes, a sample is taken from a bypass stream, filtered and measured with an RPLC-ESI-Q-Exactive HF setup. Influent and effluent of the other sites were investigated with daily composite samples and measured in the lab with an RPLC-ESI-Explorer240 setup. The high temporal resolution of the MS2Field gave unique insights into concentration dynamics of pharmaceuticals in a WWTP effluent, allowed to accurately capture maximum effluent concentrations and link concentration peaks to specific cleaning events at the production site. 11 out of 14 processed pharmaceuticals showed peak concentrations that were caused by industrial input with effluent concentrations up to 6'000 ng/L. For the other sites, industrial emissions were detected with maximum effluent concentrations up to 1'100, 460'000 and 79'000 ng/L. Although the amount of industrial wastewater is small compared to discharges from households and the loss rate from production low, emissions still make up a substantial fraction of the pharmaceutical load. Additionally, peak concentrations in the effluent can be relevant for risk assessment considerations in the receiving water. The generated knowledge aims to identify factors in the production and wastewater management that influence the amount of emissions from FPIs and hence help authorities and companies to take efficient and cost-effective measures.

3.12.T-08 Mixture Effects of Organic Contaminants in Stormwater Runoff and a Receiving Stream

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Heavy rainfalls can lead to severe flood events and environmental degradation due to discharge of contaminated stormwater runoff into surface waters. Numerous organic chemicals are used in agriculture and urban areas including pesticides, biocides, flame retardants, and corrosion inhibitors. Many of these compounds have been detected in stormwater but the assessment of runoff quality remains challenging due to highly variable stormwater composition over time and space, the vast number of often unidentified chemicals, and unknown effects of chemical mixtures. In our study, we complemented chemical analyses with cell-based *in vitro* high-throughput bioassays to shed light on (i) sources of dissolved and particle-associated organic contaminants and

their mixture toxicity in stormwater runoff, and (ii) temporal dynamics of the chemical mixtures and toxicity in a receiving stream over the course of consecutive storm events. In June 2021, we captured three storm events in the Ammer catchment (close to Tübingen, Germany) during which we collected grab samples from potential pollution sources and time-proportional composite samples from the receiving stream (the Ammer River). We screened all samples for more than 600 organic contaminants using high resolution mass spectrometry, and performed *in vitro* bioassays to assess mixture effects (cytotoxicity; activation of the estrogen and aryl hydrocarbon receptors, oxidative stress response, and algal toxicity). While during dry weather the mixture toxicity in the Ammer River was dominated by wastewater treatment plant effluent, we identified additional rain-driven input pathways of bioactive organic contaminants into the river including runoff from industrial sites and residential areas, overflows of combined sewer basins, as well as agricultural tributaries. During consecutive storm events, the chemical mixture effects in the Ammer River followed the discharge peaks indicating that storms can repeatedly mobilize bioactive compounds from surfaces leading to a significant input of both particle-associated and dissolved organic contaminants into rivers. Iceberg modeling of stormwater mixture toxicity revealed that only a small fraction of the observed effects in bioassays can be explained by the quantified chemicals highlighting the need to improve stormwater monitoring and management strategies to reduce diffuse pollution of surface waters.

3.12.T-09 Identifying Bioactive Antimicrobials and Glucocorticoids in Effluent With Effect-Directed Analysis

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Antibiotics and synthetic glucocorticoids are both pharmaceuticals that are used to treat patients that suffer from inflammation. Antibiotics are prescribed to treat bacterial infections, whereas glucocorticoids are prescribed for their pain-relieving effects resulting from inflammatory disorders. The prescription rate of these drugs is high and leads to the detection of these compounds in the aquatic environment as they are not fully removed from wastewater. Once in the environment, these compounds may adversely affect environmental and human health. In this study, we aimed to identify bioactive antibiotics and glucocorticoids – including bioactive metabolites and transformation products – in wastewater effluent from a selected group of six Dutch wastewater treatment plants by applying Effect-Directed Analysis (EDA). An antibiotics bacterial cell-based assay and the GR-CALUX bioassay were used to identify biological activity in fractionated and unfractionated sample extracts. In this EDA approach, suspect and nontarget screening techniques were applied to features related to bioactive fractions. The unfractionated extracts that were tested in the antibiotics assay showed comparable IC50 values among samples: the highest and lowest IC50 value differed a factor of 2.4 (enrichment factors of approximately 9 - 22). The activity of unfractionated extracts in the GR-CALUX assay differed by a maximum factor of 2.9 (approximately 100 – 290 ng dexamethasone equivalents/L). The response in the fractionated extracts was also comparable among samples for both assays. The antibiotics bioassay chromatogram contained three regions with bioactive fractions for five out of six samples. On average, 4 out of 80 fractions were identified as bioactive. The GR-CALUX assay identified two bioactive regions in all extracts; on average 6 out of 80 fractions were bioactive. In the presentation, we will demonstrate the annotation of features related to bioactive fractions and the assignment of identification confidence levels.

3.12.T-10 Moderated Discussion-3.12

3.12 Organic micropollutants in urban waters - occurrence, transformation, and treatment (Virtual Only)

3.12.V-01 Adsorption of Selected Polycyclic Aromatic Hydrocarbons From Contaminated Water Using Regenerable Graphene Wool

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Polycyclic aromatic hydrocarbons are ubiquitous and carcinogenic micropollutants detected in water bodies and poses threat to human health and the environment. In this research, a novel graphene wool (GW) was used as an adsorbent material for the removal of phenanthrene (PHEN) and pyrene (PYR) in aqueous solution. Adsorption kinetics, adsorption isotherms, thermodynamics of adsorption and effect of pH, ionic strength, and temperature on the adsorption of PHEN and PYR onto GW from aqueous solution were investigated comprehensively. All isotherms and kinetic experimental data were fit to Langmuir, Freundlich, Temkin, Sips and Dubinin-Radushkevich (D-R) models, and pseudo-first-order and pseudo-second-order kinetic models. The adsorption kinetic data best fit the pseudo-second-order kinetic model for PHEN and PYR sorption with R^2 value >0.999 , and adsorption increased up to 24 h. The isotherm models which best represent the data obtained were the Sips model for PHEN and PYR. The Freundlich isotherm model was found to fit experimental data better than the Langmuir model, suggesting multilayer adsorption and pore-filling mechanism would best describe GW-PHEN and GW-PYR interactions. The maximum adsorption capacity of GW for PHEN and PYR was 5 and 20 mg g⁻¹ and removal efficiency was 99.9% and 99.1% respectively. The high adsorption capacity and regenerability/reusability of GW make it a very attractive material for the removal of hydrophobic organic micro-pollutants in water.

3.12.V-02 An Update on Estrogenic Compounds in the WATER of the Douro River Estuary (Portugal)

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The quantity and distribution of seventeen endocrine disruptor chemicals (EDCs), including oestrogens, phytoestrogens, sitosterol, and banned industrial pollutants, were examined in ten locations in the Douro River estuary. Surface water samples were obtained

in 2019. The waters were filtered and subjected to solid-phase extraction (SPE) to extract and pre-concentrate (4,000-fold) the EDCs after an examination of the physicochemical data (ammonia, nitrates, nitrites, and phosphates, for example). Before being analyzed using gas chromatography-mass spectrometry, the extracts were derivatized with BSTFA and 1% TMS (GC-MS). All of the EDCs studied have a high detection rate (on average 97%), indicating that they are common in this estuary. The presence of significant physiologic levels of oestrogens (up to 9 ng/L for estradiol, E2), phytoestrogens (up to 830 ng/L for biochanin A, BIO-A), and industrial pollutants (up to 3 g/L for nonylphenol di-ethoxylated, NP2EO) in nearby aquatic species implies an endocrine disruption risk. The global concentration of E2-equivalents (E2-EQs) at this site is 25 ng/L, with a hazard quotient (HQ) of 55, suggesting a high risk of local biota harm. Furthermore, the physicochemical data indicate that direct sewage flows, most likely from tourist boats, might cause injury. Finally, aside from their impacts on fauna, the presence of all studied components may have an impact on human health if they come into direct (fluvial beaches) or indirect (food) contact with the tested chemicals. Funding: Project ATLANTIDA (NORTE-01-0145-FEDER-000040), by NORTE 2020, under PORTUGAL 2020, through ERDF); FCT and ERDF UIDB/04423/2020 and UIDP/04423/2020; and ICBAS.

3.12.V-03 Carbon-Covered Alumina Supported ZnO Nanocatalysts With Enhanced Visible-Light Photocatalytic Performance for the Removal of Azo Dyes

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In this study, economical and green catalysts were prepared as a positive step in the direct use of solar light for the degradation of wastewater polluted with industrial dyes. Hence, zinc oxide (ZnO) nanocatalysts were prepared by the sol-gel method using zinc acetate as the precursor and successfully embedded on carbon-covered alumina (CCA) supports. The catalysts were characterized by a variety of techniques that include XRD, HR-TEM, EIS and BET. All the new CCA-implanted catalysts were crystalline with high surface areas. The photocatalytic activities of all the catalysts were fully studied using tartrazine and sunset yellow azo dyes as model pollutants. In addition, full kinetics and mechanistic details are presented and analyzed.

3.12.V-04 Occurrences and Ecological Risks of Tamiflu Metabolites in Treated Wastewater in Taiwan

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Humans highly use antiviral drugs Tamiflu during the flu season. The excreted active Tamiflu metabolites pass through wastewater treatment then discharge into rivers, which has sparked environmental concern potential ecological risks. This study aims to assess ecological risks posed by Tamiflu metabolites, oseltamivir ethylester (OE), and oseltamivir carboxylate (OC) of treated water in wastewater treatment plants (WWTPs) in Kaohsiung, Taiwan. We collected wastewater from primary and tertiary WWTPs during the flu season to detect the presence concentration of Tamiflu metabolites throughout the processing system of wastewater treatment and determine the removal efficiency. There is 100% OC detection in the tertiary WWTP, whereas only one-third of the influent and effluent samples can be detected OC in the primary WWTP. Major metabolite product OE was detected in all process systems in both WWTPs. Both WWTPs have low removal efficiency for Tamiflu metabolites, especially for OE. Surprisingly, the performance of tertiary WWTP is not significantly better than primary WWTP for removing Tamiflu metabolites. There are no ecological risk concerns to Tamiflu metabolites through treated water and rivers, even though highly frequency detection in OC and low removal efficiency.

3.12 Organic micropollutants in urban waters - occurrence, transformation, and treatment (Poster)

3.12.P-Th082 Stable Isotope Labelling With Heavy Ozone for Detection of Ozonation By-Products in Effluent Organic Matter

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Introduction Ozonation is a chemical oxidation method for the treatment of wastewater to enhance removal of contaminants. While lots of research has been conducted regarding the transformation of organic micropollutants with ozone, effluent organic matter (EfOM) is not well studied in terms of ozonation by-products (OBPs). Since EfOM is a major consumer of ozone, it is critical we understand the OBPs produced from EfOM. Ultra-high resolution mass spectrometry like FT-ICR-MS is the primary method for non-target analysis of complex mixtures like EfOM, but OBP detection is still limited due to complexity. With stable isotopically labeled heavy oxygen, labeled ozone can now be generated and reacted with EfOM to produce isotopically labeled OBPs. This mass label can be used to unambiguously identify OBPs formed from EfOM as well as contaminants of concern. By examining the ratio of produced OBPs with 18-O and 16-O formulas, we can better understand the reactions producing these OBPs. Since the ratio between labeled and non-labeled oxygen in our ozone generator can be controlled, we can monitor the production rate of the 18/16-O OBPs produced. By using model compounds, we can leverage this conserved ratio to better understand the OBPs found in EfOM. **Methods** Extracted EfOM was ozonated with 18-O mass labeled ozone. The custom ozone generator uses recirculation of 18-O heavy oxygen to produce mass labeled ozone. After ozonation, EfOM was measured using FT-ICR-MS to detect OBPs. **Results** The ozone generator successfully created 18-O labeled OBPs based on model compounds used with known ozone reaction mechanisms. OBPs from venlafaxine and sulfamethoxazole were produced with this heavy ozone and the mass labeled products detected. Over 2,000 OBPs with mass labeled oxygen can be detected in 18-O ozonated EfOM. All of these formulas are in a series with increasing numbers of 18-O (up to 5) found in the molecular formula. About 1,700 unique series are detected, confirming that these formulas are indeed generated through ozonation. Based on the purity of 18-O in the

system, a ratio between the isotopically labeled OBPs and the non-labeled OBPs is consistent for model compounds. In our experiment with 40% heavy oxygen in the system, venlafaxine produced an 18/16-O ratio of 0.78 for the primary ozonation product (venlafaxine-n-oxide). This ratio can be used to better validate 18-O labeled OBPs found in EfOM.

3.12.P-Th083 Physico-Chemical Properties and Environmental Fate Predictions of Dienogest and Its Transformation Products

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Dienogest is synthetic steroid hormone widely used for human contraception. As of such, dienogest is commonly detected in surface waters near wastewater treatment plants (WWTPs), which is of concern due to dienogest's potential as an endocrine disrupting chemical. Due to the tendency of dienogest to transform in the environment, the environmental behaviour of 27 previously identified transformation products of dienogest were also investigated in this study. Primarily, two different transformation processes are examined in detail: metabolism and phototransformation. As many of the physico-chemical properties of dienogest and its metabolites have not yet been experimentally characterized, COSMO-RS solvation theory was used to estimate the key fate-determining properties of these compounds. Specifically, we report here estimates of vapour pressure, melting point, solubility in water, various environmental partitioning ratios, and atmospheric degradation rate constants for hydroxyl removal. The environmental fate and persistence of dienogest and its transformation products were then estimated using fugacity-based modelling. Using the fugacity-based Equilibrium Criterion (EQC) model, the chemicals are shown to remain in water with minimal partitioning into sediment, soil, and air, if released to water. The results of the fugacity-based modelling were examined in detail with respect to the structural modifications that occur during the transformation processes. This study provides a preliminary overview of the environmental fate and persistence of dienogest and its transformation products which can be used to inform future risk assessments.

3.12.P-Th084 Evaluation of Cytogenotoxic Potential and Embryotoxicity of KrishnaRaja Sagara (KRS) Cauvery River Water in Zebrafish (*Danio rerio*)

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*Polluted water is a major concern in our daily lives. Humans indiscriminately dumped thousands of chemicals and waste materials into the water, most of which are unknown and lack adequate toxicity data. The Cauvery River (CR), an interstate river in South India, show the presence of *Cyclops*, *Daphnia*, *Spirogyra*, and/or *Spirochaeta* which are bioindicators of water pollution at the three study stations (fast-flowing water [X], slow-flowing water [Y], and stagnant water [Z]). Total coliform and *E. coli* which are indicators of sewage contaminants by animals or humans were also detected. All physicochemical parameters tested, including heavy metals, were within the limit acceptable by standard organizations except for DO, BOD, and COD, which indicate hypoxic water conditions. As a result, both water samples and zebrafish larvae had significantly less oxygen when tested using SEM/EDS. Furthermore, for the first time in any water sample analysis globally, we discovered the presence of microplastics (polybutene, polyisobutene, and polymethylpentene) and cyclohexyl in CR water samples using Raman spectroscopy. Acute treatment of zebrafish (*Danio rerio*) embryos with the water samples generated reactive oxygen species (ROS), which triggered subcellular organelle dysfunctions, skeletal deformities, pericardial edema, and increased mortality. How pathogens/microplastics enter the aquatic environment and human food chain is critical for future prevention.*Anifowoshe AT, Roy D, Dutta S, Nongthomba U. Evaluation of Cytogenotoxic potential and Embryotoxicity of KRS- Cauvery River Water in Zebrafish (*Danio rerio*). **Currently Under review in Ecotoxicology and Environmental Safety.**

3.12.P-Th085 Facing Urban Contamination: Detection and Quantification of Organic Contaminants in Urban Water From Barcelona City (Spain) Using Lc-Hrms/Ms Orbitrap

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Recently, municipalities have started developing and implementing stormwater and watershed management plans monitoring stormwater pollutants such as pathogens, nutrients, total suspended solids, toxic metals, and organic contaminants, e.g., polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and certain pesticides and plasticizers. Most of these monitoring programs target priority regulated compounds, but the majority of compounds and their transformation products remain to be discovered and lack rigorous toxicity information. Although large efforts have been made in the last two decades to identify novel contaminants and their TPs, taking advantages of innovative analytical methodologies for specific compounds classes, an unbiased non-targeted approach is considered a viable tool for comprehensive characterization for stormwater contaminants. Nevertheless, last advances in Analytical instrumentation have enabled their rapid, accurate detection and quantification. Under this context, High-Resolution Mass Spectrometry is considered the most promising instrumentation, even if analytical standards are not available, to close the knowledge gap. Several studies have reported their occurrence in different environmental compartments (surface and groundwaters), and they have potential to cause adverse effects on human and environmental health. Here, the recollected urban water was first filtered under vacuum at 0,7 µm employing glass fiber filters and next, 500 mL of water was extracted by means of a Solid-phase extraction using a home-made cartridge containing four different sorbents: Hydrophilic-Lipophilic Balanced, weak anion exchange, weak cation exchange and priority pollutants. Then, cartridges were

eluted with a mixture of ethyl acetate and methanol using 5% ammonia and 2% formic acid in independent steps. Finally, the elution mixture was evaporated under gentle N₂ and reconstituted. Results prove the presence of organic contaminants in urban groundwater however, the majority of them reveals a low concentration in the aquifers. It is worth to mention that compounds with a low Log P showed more frequency of detection and concentration than the non-polar compounds studied. It has been demonstrated that organic pollutants are present in urban water from Barcelona. Persistent polar compounds were the main detected as they are unable to accumulate in soil, hence bypassing natural barriers ending in aquifers.

3.12.P-Th086 In-Stream Monitoring of Combined Sewer Overflow Impact Using a Combination of Continuous Probes and Passive Samplers

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Combined sewer overflows can impact surface waters with classical organic pollution leading to oxygen deficits or ammonia exceedances but do also carry important loads of biocides and untreated micropollutants. These impacts are rarely monitored in urban settings with multiple potential outlets. Planning of retention structures in combined sewer systems are often based on theoretical hydraulic calculations/simulation without any pollutant load validation. Their true efficiency is therefore often largely unknown. This project gained quantitative information on combined sewer overflows via continuous in-stream measurement with probes in longitudinal profiles. The probes were mounted in an urban stream with respect to expected inputs by sewer systems. Two main parameters were used to detect discharged overflow loads: ammonium via an ion selective electrode and oxygen via membrane fluorescence detection. Ammonium proved to be reliable and easier to interpret because of its slower decay in surface waters. Within the 3 km observed it was relatively stable and hence fit for quantification of loads while oxygen often hit zero in-stream levels and was also strongly influenced by reaeration at high flow. Nevertheless, it was useful in making qualitative interpretations of overflow sources and corresponding locations. The project also introduced passive samplers as complementary method to detect overflow discharges on the basis of pharmaceutical and biocide emissions. Passive samplers are successful in a certain range where dilution by very large flood waves is not too important, but concentration shifts are yet distinguishable from baseflow (given the shorter exposure to flood waves). All in all, the techniques showed promise to assess the impact of overflows on surface waters and the pinpointing of areas of discharge, although overflow structures were too numerous and too close-range to be differentiated within one observed segment. Combined immission measurements and sewer modelling might be an interesting approach to improve the techniques.

3.12.P-Th087 The Pathway of Quaternary Ammonium Biocides to Surface Waters - an Environmental Forensics Approach

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Quaternary ammonium compounds (QUATs) are surface-active, antimicrobial, high production volume chemicals with a broad range of application from agriculture or industrial processes to consumer products, like fabrics softeners, cleaning agent, disinfectant, preservatives and personal care products. QUATs are salts of positively charged polyatomic ions of the structure NR₄⁺, R being as alkyl group or an aryl group. Differences in the alkyl part lead to differences in classes of compounds such as: alkyldimethylbenzyl ammonium chlorides (BACs) with C8-18 alkyl groups; didecyldimethylammonium chlorides (DDACs) with C8-20 alkyl groups; tetradodecyl-ammonium chlorides (TDACs); alkyltrimethyl ammonium chlorides (ATMACs) with C10-C22 alkyl groups. This contribution aims to identify the different sources and inputs of QUATs for Danish surface waters. For this purpose, we linked the QUAT concentrations in surface waters to input from WWTPs, using the concentration of benzotriazole as a marker of the wastewater fraction in the respective water body. Both, Quaternary Ammonium Biocides and benzotriazoles were quantified by means of HPLC-MS/MS with direct injections after carefully removing all internal contamination of QUATs from the instrument. Each geographical data point is represented by duplicate samples to enable identifying contamination issues. Concentrations of QUATs were remarkable especially for BAC 12, 14 and 16, which ranged from 10 ng/L to 20.000 ng/L. While different WWTPs clearly contributed to the concentrations of BAC 12, 14 and 16 and were in selected parts of the rivers the only or dominant source, the highest concentrations (up to 10.000 ng/L) in surface waters originated from other sources. One other source was only active under rainy weather, corresponding to the suspected source: surface water runoff (e.g., roof runoff, as roofs are frequently treated with benzalkonium chlorides in Denmark). Another source, giving high inputs, was active under dry weather and could not yet be contextualized.

3.12.P-Th088 Difference in Reaction Pathways of Tramadol and Venlafaxine N-Oxide in Pure Water and Effluent Wastewater During Ozonation

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Advanced treatment technologies such as ozonation, activated carbon, membrane bioreactor, etc. have shown promising results in removing organic micropollutants like pharmaceuticals from wastewater. Ozonation can reduce the load by reacting pharmaceuticals with ozone and hydroxyl radicals that are formed during the technical ozonation process. While removing compounds they often get transformed to other products. Tramadol and Venlafaxine N-oxide are primary ozonation products of Tramadol and Venlafaxine. However, they react further in the presence of excess ozone. This study aims to explore the difference in yield of transformation products of Tramadol and Venlafaxine N-oxide due to different ozone dosing approach and the effect of water matrix. Most mechanistic studies are conducted in pure water. However, in this study we show that both removal and reaction pathways are significantly different in pure water compared to effluent wastewater. This is including the fact that the

yield of transformation products resulting from direct reaction with ozone is lower in effluent wastewater than pure water. Conversely, the yield of transformation products resulting from reaction with OH radical is higher in effluent wastewater.

3.12.P-Th089 Germany-Wide Biocides Monitoring in Sewage Treatment Plants

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The impact of biocides on the environment has increased in recent decades due to their increased use. In individual studies, biocidal active substances have been detected in many environmental compartments. However, as an evaluation of the German Environment Agency (UBA) shows, there is currently no representative picture of the actual environmental pollution beyond individual findings in Germany. One reason for this, is the fact that no systematic environmental monitoring has been carried out in Germany to date in relation to biocides. Therefore, this waste water project was initiated, which systematically investigated prioritised biocidal substances and transformation products in the influent and effluent of sewage treatment plants (STP), primary and excess sludge, as well as stormwater discharges and combined sewer overflows (CSO) over a period of one year. A total of 26 biocides (disinfectants, material preservatives and pest control products) were analysed in effluents of 29 STPs distributed over the entire federal territory (three of them purely served by a separate sewer system). Additionally, influent and sludge of five STPs, the discharge of two stormwater clarifiers and the discharge of six CSO tanks were investigated. Finally, more than 450 samples were analysed for biocides, but also typical sewage parameters for checking the operating condition of the STPs. Most biocides investigated were detected in a large number of samples above the analytical limit of detection. Some substances also exceeded the environmental quality standard (EQS) or the predicted no effect concentration (PNEC) for surface water. With the project results, for the first time a reliable nationwide statement is possible for a larger number of biocides regarding emissions from STPs into surface waters. A further ongoing project focusses on CSO discharges for receiving more insights on the importance of this little investigated entry path.

3.12.P-Th090 Nontarget Analysis of Polluted Surface Waters: A Case Study in Bangladesh

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In Bangladesh, the high density of population and industry, as well intensity of agriculture practices, leads to major surface water contamination, including by organic micropollutants. Given that Bangladesh's population also depends on local fish as a staple dietary component, this contamination poses unknown risk to both human and environmental health, thus here we undertook a surface water monitoring survey with comprehensive analysis by nontarget mass spectrometry. Surface waters were sampled from 12 sites located on 4 different rivers around the capital city, Dhaka. Samples were spiked with internal standards and filtered before analysis by liquid chromatography high-resolution mass spectrometry in 4 modes: positive and negative electrospray (\pm ESI), and atmospheric pressure chemical ionization (\pm APCI). Among 39,030 total detected molecular features, there was relatively little redundancy between modes, and over 26,000 unique features were detected. Given that 51.9-77.5% of the features in each mode were unique, the combination of these 4 ionization modes is necessary to cover the relevant chemical space in polluted surface waters. In a principal component analysis, components PC1 and PC2 explained 54.8% and 6.4% of the overall data variability, respectively, and the scores plot revealed two groups of samples separated by geographical location on PC1, and an upstream-downstream distribution on PC2. This suggested strong chemical profile differences between highly urbanized and more remote sampling sites, and a pollution gradient along the flowpath of rivers moving towards the Bay of Bengal. A nontarget workflow using open science tools allowed us to confirm or annotate many anthropogenic contaminants, including pharmaceuticals (e.g. diclofenac), pesticides (e.g. diuron), and industrial chemicals (e.g. anionic surfactants). An example annotation that may be related to local textile industry was indigo dye (level 2a, [4]), which had a high MS² similarity score (796/1000) compared to the public MS² database available on MS-DIAL website (v2021/04/13, <http://prime.psc.riken.jp/comprms/msdial/main.html>). Most compounds identified had a spatial distribution with higher response intensity closer to Dhaka. Work is ongoing to identify contaminants at higher confidence levels, their likely sources, and bioaccumulation in fish sold at local markets

3.12.P-Th091 Evaluation of the Efficiency of a New Secondary Biological Treatment Based on Ultrafiltration Membranes in Galindo Wastewater Treatment Plant

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Wastewater treatment plants (WWTPs) are one of the major secondary sources of contaminants of emerging concern (CECs) to the environment, since conventional treatments implemented are not efficient enough for their complete removal. The objective of the present work was the analysis of the efficiency of a new wastewater secondary treatment based on membrane bioreactors. In this sense, the application of a multiresidue analysis of up to 337 emerging and priority contaminants ($\log K_{ow} = -4.2-6.8$) including 50 semi-volatile organic compounds (SVOCs), as well as a suspect screening for "unknown" compounds identification in different types of effluents, were the strategies implemented. Composite samples (24 h) of three WWTP effluents (one primary and two different secondary treatments, a conventional Biological Mix and another using ultrafiltration membranes) were taken during 9 weeks (February-May 2021) from Galindo WWTP located in Sestao, Biscay, Spain. Samples were preconcentrated by means of solid phase extraction (SPE) protocol using in-house prepared triphasic cartridges (Strata HR-X:ZT-WAX:ZT-WCX,

3:1:1). Cartridges elution was performed with two MeOH:EtOAc (1:1, v:v) solutions, the first containing 2% ammonia (v/v), and the other 1.7% (v/v) formic acid in the case of more polar compounds, whereas SVOCs were eluted with 12 mL of hexane:toluene (4:1, v:v). The detection of the contaminants was carried out by means of an ultrahigh performance liquid chromatograph coupled to a high resolution mass spectrometer q-Orbitrap (UHPLC-qOrbitrap) (Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific™ Q Exactive™ Focus quadrupole-Orbitrap mass spectrometer, Thermo, CA, USA) and gas chromatograph coupled to a quadrupole mass spectrometer (GC-MS) (Agilent 6890 gas chromatograph coupled to an Agilent 5975 mass spectrometer system, Agilent Technologies, Palo Alto, USA). For the suspect analysis the NORMAN database including more than 40000 compounds (www.norman-network.net) was used as suspect list as well as applying the constraint of being included in mzCloud database (www.mzcloud.org). It is necessary to emphasize that the application of a biological treatment with an ultrafiltration membrane seems do not improve the expectations of this type of WWTP treatments compared with the current conventional biological ones applied at the Galindo WWTP since several compounds used as efficiency indicators for new implemented treatments including carbamazepine, diclofenac or sulfamethoxazole were found at similar concentrations in both secondary treatments effluents.

3.12.P-Th092 Primary Degradation of Benzophenone-Type UV Filters in Lab Experiments and Their Structure-Related Endocrine Activity

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Trace organic contaminants (TrOCs) are a diverse and expanding area of anthropogenic substances with possible dangerous effects on ecosystems and human health. One important group of TrOCs that have been getting more attention in research over the last years are benzophenone (BP)-type UV filters. As most UV filters are photostable and relatively hydrophobic, they tend to bioaccumulate in biota and soils or sediments with high organic content act as natural sinks. Residues have been detected in various aqueous matrices as well as in fish, possibly reaching humans due to bioaccumulation. Furthermore, BP-3 and its transformation product, BP-1, have been found in human urine after application of personal health care products to the skin. Some derivatives such as BP-1, BP-2, BP-3, and BP-8 have already been linked to coral bleaching and the structural similarity of BPs to estradiol links them to a potential endocrine activity which has been demonstrated in vivo and in vitro. Removal experiments have been conducted with a duration of two weeks with BP, BP-1, BP-2, BP-3, BP-4, BP-6, BP-7, BP-8, and BP-10 spiked into river water at mass concentration $c = 1 \text{ } \mu\text{g/L}$. Trace analysis of BPs was carried out via UHPLC system (Nexera, Shimadzu) in combination with a tandem mass spectrometer (QTRAP 6500+, Sciex) according to a method developed at the institute. Removal of BPs under suboxic conditions (dissolved oxygen $< 1 \text{ mg/L}$) on pumice simulating soil passage during river bank filtration was investigated under different conditions. Primary decay of more than 50 % was observed for all BPs at pH 7 under biotic conditions. High and fast removal indicated by half-lives ($t_{1/2}$) of only 2 h was detected for BP-2, BP-1, as well as for BP-7, BP-10 and BP with $t_{1/2} < 12 \text{ h}$. Removal rates have been calculated by using the first order exponential decay fit. Hydrophobic BPs with estimated log KOC > 3.1 , namely, BP-10, BP-6, and BP-7, showed high removal (60-90 %) in abiotic experiments at pH 7, while BP-3 and BP-8 with lower estimated log KOC were removed less efficiently (50 %). However, BP-4 was removed very poorly due to its low sorption affinity. Structure-activity relationship rules regarding endocrine activity using yeast receptor gene assays could be confirmed for several BPs. High estrogenic activity was exhibited by BP-1 and BP-2 due to hydroxyl groups in para and ortho positions. Other substituents such as methoxy and chloride might have diminished the effect.

3.12.P-Th093 Organic Micropollutant Removal From Urban Waters by Nature-Based Solutions: Introduction to MULTISOURCE Project

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MULTISOURCE is a starting up EU Horizon 2020 project “ModULar Tools for Integrating enhanced natural treatment SOLUTIONs in URban water CyclEs”. The project operates seven pilot plants of nature-based solutions treating wastewater, urban runoff and rainwater in France (INRAE), Belgium (Rietland), Italy (IRIDRA), Spain (ICRA), Norway (NIVA), Germany (UFZ) and USA (Montana State University). These nature-based solutions comprise green roofs, green walls, raingardens and constructed wetlands derived eco-technologies. These systems, besides water management, provide practical and aesthetic benefits and are currently under intense research. MULTISOURCE will demonstrate operation efficacy of the seven different pilot plants for seven different water types. Besides, it will develop innovative tools, methods and business models to support the technology upscale, assimilation and long-term maintenance. The pilot plants are integrated in urban infrastructure like building walls and parking lots and provide great potential for water reuse. In order to realize this potential, the installations must remove pathogens, nutrients, hazardous organic micropollutants and contaminants of emerging concern, including microplastics. MULTISOURCE will assess the occurrence and fate of the water contaminants within and beyond the current regulatory framework. We are particularly interested in the pilot efficiency for the micropollutant removal, which will be assessed in cooperation with the different project partners, including variations due to e.g. non-baseline operations and seasonality. For that, we are implementing several different analytical methods for suspect-screening and target analysis of over 300 relevant organic compounds. These include active pharmaceutical ingredients, pesticides, surfactants, drugs of abuse, petroleum constituents and combustion products and transformation products of some of these compound groups. Harmonised monitoring plan and tailored approach for each pilot ensuring comparability of the different infrastructures and water types has been developed. The first results of the micropollutant content and the pilot capacity for their treatment will be shown at the SETAC conference. The results will be further used for the planned environmental risk analyses and assessment of water reuse potential.

3.12.P-Th094 Occurrence and Mass Flows of Contaminants of Emerging Concern (CECs) in Sweden's Three Largest Lakes and Their Connected Rivers

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Contaminants of emerging concern (CECs) are of concern for the aquatic environment due to their possible adverse effects on the environment and humans. The overall aim of this study was to assess the occurrence and mass flows of CECs in Sweden's three largest lakes and their connected rivers. This study is the first of its kind in Sweden to systematically study CECs in the three largest lakes in Sweden. The occurrence and distribution of 112 CECs was investigated, including 73 pharmaceuticals, 13 perfluoroalkyl substances (PFASs), 8 industrial chemicals, 4 personal care products (PCPs), 3 parabens, 2 pesticides, and 4 other contaminants (mostly anthropogenic markers). This is one of the first studies reporting environmental concentrations of, for example, the industrial chemicals tributyl citrate acetate and 2,2'-dimorpholinyl-diethyl-ether. The CEC concentrations in river surface water were generally higher (31–5200 ng/L; median 440 ng/L) compared to lake locations (36–900 ng/L; median 190 ng/L). At urban lake sites, seasonal trends occurred for PCPs and parabens as well as for antihistamines, antidiabetics, antineoplastic agents, antibiotics, and fungicides. The median mass load of the rivers was 180 g/day (4.0–4300 g/day) with total mass loads of 5600 g/day to Lake Mälaren, 510 g/day to Lake Vättern and 5000 g/day to Lake Vänern. All three investigated lakes are used as drinking water reservoirs and further investigations of the impact of CECs on the ecosystem and human health are needed.

3.12.P-Th095 Investigation of a Multi-Stage Process for Wastewater Reclamation With Special FOCUS on Micropollutant Removal

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New concepts for nutrient reclamation from wastewater through production of a liquid fertilizer can help to reduce agricultural irrigation water demand in water-scarce countries, as well as decrease micropollutant burden on treatment systems. However, it's most beneficial for reducing mineral fertilizer consumption. For reuse of nutrient-rich centrate water – side-product from sewage sludge treatment – a combined treatment process including a membrane-bio-reactor (MBR) and an electro-dialysis (ED) membrane stack is envisioned as a multi-barrier concept for the abatement of plant-available micropollutants. Using a lab-scale setup, real wastewater was treated with this multi-stage process over the course of several months. Samples were analysed with LCMS targeting a set of 29 polar organic micropollutants across different molecular properties which potentially influence plant uptake such as molecular weight and pH-dependent polarity. Furthermore, ICP-MS/OES analysis were performed to monitor both nutrient dynamics as well as inorganic contaminant's removal. Results show that removal rates of the combined process for many investigated compounds such as diclofenac or gabapentin exceeded 90%. While the MBR is able to remove biodegradable compounds well, an increase in concentration of biological transformation products such as gabapentin-lactam was observed. Acting as a second treatment stage, the ED shows a median removal of >90% across all investigated compounds. Overall, experiments show that the investigated multi-stage process is able to produce a nutrient solution that meets legislative requirements of the EU for wastewater reuse. Additionally, by reducing the micropollutant burden in wastewater treatment plants, the investigated process can be integrated into a holistic future concept for wastewater treatment plants.

3.12.P-Th096 Cytotoxicity As a Proxy for Particle-Associated and Dissolved Organic Contaminant Loads in Rivers

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Storm events lead to a mobilization of particulate matter, dissolved and particle-associated organic pollutants that pose a risk to river ecosystems. Target screening can hardly capture the broad range of compounds present in stormwater and receiving streams. Thus, an additional monitoring proxy that describes the overall chemical load in stormwater is needed. Each chemical in a mixture contributes, albeit with different potency, to cytotoxicity measured by reduction of cell viability after 24h in four human cell lines. Thus, the aim of this study was to investigate the applicability of cytotoxicity as a proxy for the organic contaminant load of rivers during storm events. Field investigations took place in the Ammer River (annual average discharge 0.87 m³ s⁻¹) close to Tübingen, Germany, during intense precipitation events in June 2021. The sampling site was located at the outlet of the gauged catchment (134 km²), thus, integrating inflowing water from all upstream tributaries and sewer overflows. During storm events, high-resolution temporal monitoring of discharge, suspended particles, particle characteristics, as well as of dissolved and particle-associated organic contaminants was conducted using both chemical analyses and cell-based *in vitro* bioassays. The cytotoxicity in the water phase (expressed as toxic units, TU), was similar among the cell lines. TU values followed the course of the hydrograph with highest TU values at the maximum or slightly after the discharge peak. This finding suggests that the chemical load is controlled by the transported volume of water despite the fact that different contaminant sources are likely to contribute to the water flux and pollutant load in the river at different time points of the hydrograph. For the particle-associated cytotoxicity, TU values are constant over the course of the events suggesting that the particle-associated cytotoxicity in the river is, similar to the water cytotoxicity, controlled by the particle load in the river. This highlights that the cytotoxicity is a suitable proxy to detect mixtures of organic compounds and, thus, assess the chemical load in rivers during storm events.

3.12.P-Th097 Organic Micropollutants: A CASE Study on Influent and Effluent Waters in Australia

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Even though recycled water is becoming an increasingly valuable resource in a drying climate, there remains concerns about contamination with emerging contaminants. A current limitation of recycled waters is that wastewater treatment plants (WWTP) are not designed to treat organic micropollutants. As a result, the concentration, and risks of organic micropollutants is not well understood. This study evaluates pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in influent and effluent water across 31 wastewater treatment plants (WWTP) in Victoria, Australia. This work had four specific aims: (1) quantify the type and concentration of PPCPs and EDCs in influent and effluents; (2) investigate removal efficiencies of treatment technologies; (3) examine toxicity using a receptor localisation assay; (4) conduct an extensive risk assessment. Influent and effluent water was sampled from 31 WWTPs in April–May 2021. Water samples were collected using passive samplers (polar organic chemical integrative sampler ‘POCIS’ 28 d deployment), and grab samples (24h composite). Samples were screened for 72 PPCPs and 21 EDCs. As a proxy for EDC-activity, we tested if effluent waters elicit changes in nuclear localisation of androgen or estrogen receptors, and expression of their respective target genes. We found that PPCPs concentrations in influent ranged from < 0.2–2,900 µg/L, and in effluent from < 0.02–8.1 µg/L. For EDCs, concentrations in influent waters ranged from < 0.02–12 µg/L and in effluent from < 0.001–0.54 µg/L. Of the 72 PPCPs, 48 were detected in grab samples and 70 in passive samplers. In influent, codeine was detected in 100% influent samples, while a further 16 PPCPs were detected in >90% of samples. In effluent, the most frequently detected PPCPs were lamotrigine, carbamazepine, amidotrizoate, oxazepam, and acesulfame K. We found that removal rates for PPCPs were compound specific. For example, paracetamol, caffeine and acesulfame K were all removed at a mean rate of >99, 99 and 97%, while carbamazepine and venlafaxine showed limited removal (mean rate 19–61%). Furthermore, for lamotrigine, there was no evidence of removal (-28%). In the receptor localisation assay, influent samples activated the estrogen receptor and induced expression of estrogen receptor target genes, demonstrating EDC-activity. Our study underlines the importance of multiple lines of evidence approach for risk assessment of organic micropollutants.

3.12.P-Th098 Urban or Agricultural Usage As Source of Widespread Groundwater Contamination With the Fungicidal Degradation Product Nn-Dimethylsulfamide

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Groundwater monitoring has revealed a widespread occurrence of *N,N*-dimethylsulfamide (DMS) in Danish groundwater. In 2020, DMS was detected in 33% of drinking water abstraction wells, exceeding 0.1 µg/L in 9% of the wells. DMS is a degradation product of the two fungicides tolylfluanid and dichlofluanid. In Denmark, these fungicides have been used in fruit orchards and strawberry fields from early 1970’s until 2007. In addition, they have been widely used in paint and wood preservatives until 2015. Urban use has not previously been expected to cause widespread pollution in aquifers, but the geographical distribution of contamination and the relative amounts of the fungicides used as biocide and pesticide suggest that the urban use could be of importance. We made a detailed investigation of DMS occurrence in soil, drainage systems and shallow groundwater in two model areas, where DMS exceeds the drinking water standard in nearby wells used for drinking water abstraction. One of the areas is dominated by wooden buildings (garden allotments) and one is dominated by agriculture, including historical strawberry fields. The analyses revealed the presence of the fungicides in soil at both areas, though highest in soil next to wooden buildings. DMS was found above the threshold limit for drinking water in both the drainage and shallow groundwater of both areas, confirming the presence of local sources. Hence urban environments seem to be equally important as a source for groundwater contamination with DMS as agricultural environments. Sorption and degradation data show that DMS hardly sorbs to soil and that it is recalcitrant in subsoil and at anoxic conditions. DMS is therefore likely to be widely present in Danish groundwater for several decades.

3.12.P-Th099 Assessing the Biodegradation of Water-Soluble Polymers in Wastewater Systems

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While plastic materials have been one of the defining topics in environmental research in the last decade, very few studies focused on the environmental fate of water-soluble polymers. However, water-soluble polymers are used in large volumes across a wide range of applications, including home- and personal care products of which a large fraction enters the wastewater system and - depending on their persistence and removal - might end up in natural systems. In our work, we investigate the aerobic biodegradability and biotransformation of water-soluble polymers that are considered promising alternatives to conventionally used persistent water-soluble polymers. Thereby, we focus on polymer classes with structural similarities to naturally occurring macromolecules such as poly(amino acids) and modified polysaccharides, which we expect to be susceptible to breakdown by enzymes present in the wastewater. As the initial extracellular breakdown of polymers is considered the rate-limiting step in polymer biodegradation, we attempted to improve established respirometric polymer biodegradation testing schemes by focusing on the role of extracellular enzymes. For example, we assessed the effect of an incubation of the polymers with extracellular wastewater enzymes on the mineralization kinetics of polymers by wastewater microorganisms. Furthermore, we found that a prolonged pre-conditioning of the biological inoculum prior to polymer addition, which is often done in established testing schemes, might not be needed and can even be detrimental in the context of realistic experimental conditions. Given that polymer biodegradation rates can strongly depend on the presence of additional substrates, we additionally investigated the effects of co-substrates on the biodegradation kinetics of water-soluble polymers. Overall, this contribution presents ways towards more

realistic laboratory experiments for the assessment of water-soluble polymer biodegradation in wastewater and offers novel insights into the biodegradation process of water-soluble polymers in wastewater.

3.12.P-Th100 Occurrence and Distribution of Pharmaceuticals and Identification of Photo-Transformation Products in Intermittent Rivers

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Due to climate change and population growth, treated wastewater has an increasing influence on intermittent streams and can even account as the main water input during most of the year. Besides, conventional wastewater treatment plants (WWTPs) barely remove organic contaminants such as pharmaceuticals, which consequently reach the aquatic system spreading their adverse effects on the aquatic environment and human health. Pharmaceutical residues have been frequently detected in river samples at concentrations of ng L⁻¹ or even higher. However, their concentration along the river may change due to natural attenuation processes. Once pharmaceuticals are in the environment, physico-chemical phenomena can occur such as hydrolysis or photolysis and consequently transformation products (TPs) are produced. TPs can sometimes result in greater toxicity than their precursors, thus, the study and identification of TPs is essential to assess the health of a watercourse. In this study, we aim to examine the presence of pharmaceuticals in different intermittent rivers, which are dominated by the wastewater effluent, and to study their fate and the possible natural attenuation. Surface water samples from the Osona region in Spain were sampled during the different seasons 2020-2021 and different sampling points were collected; upstream, the WWTP discharge point (DP), 20m after the WWTP DP and between 150m and 250m after the WWTP DP. All samples were extracted through a Solid Phase Extraction (SPE) using a homemade multilayer mix-bed SPE cartridge containing four different specific sorbents covering a wide range of polarities. Besides, lab-scale photolysis experiments were also performed in order to explore probable photo-TPs to be included in a suspect screening database to check their presence in the surface water samples studied. Target analysis for more than 100 pharmaceuticals was performed using liquid chromatography high-resolution mass spectrometry Q-Exactive Orbitrap. Over 50 pharmaceuticals were found and quantified in all six studied streams. Concerning lab-scale photolysis, around 10 photo-TPs were elucidated while only one photo-TP was found in the water samples. The detection of photo-TP provides evidence of photolysis and proves the importance of natural attenuation processes in rivers.

3.12.P-Th101 Mixture Effects of Organic Chemicals in a Highway Runoff Retention Pond

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Highway runoff contains complex contaminant mixtures that can pose risks to aquatic ecosystems and drinking water resources. Stormwater retention ponds are designed to store and treat runoff prior to discharge into surface waters with particular focus on particle removal. However, in addition to particle-associated pollution, highway runoff carries dissolved and mobile contaminants, which may not be removed well in conventional retention basins. Furthermore, numerous chemicals in highway runoff remain unknown but likely contribute to mixture toxicity. During an intense storm event in February 2021, we sampled highway runoff from a motorway in Germany (close to Tübingen, Germany) and tracked the dissolved and particle-associated chemical and toxicological profile of the runoff throughout a stormwater retention pond, which discharges into a stream. Sample treatment involved solid phase extraction to enrich dissolved contaminants as well as accelerated solvent extraction to extract contaminants from suspended particles and pond sediment. We screened all samples for more than 600 organic contaminants using high resolution mass spectrometry and performed several *in vitro* bioassays to assess mixture effects. While the highway runoff did not trigger any estrogenic activity nor neurotoxicity, we observed high cytotoxicity, activation of the aryl hydrocarbon receptor, and unexpectedly high binding to the peroxisome proliferator-activated receptor gamma. The observed effects in highway runoff were constant throughout the stormwater retention pond indicating low removal of both dissolved and particle-associated contaminants. In fact, numerous motor vehicle-related organic compounds such as benzothiazole and pyrene were detected in the influent and effluent of the stormwater retention pond with little or no decline in the concentration of the chemicals. The discharge of the stormwater retention pond contributed to the overall contaminant load in the receiving stream, which had a lower cytotoxicity than the pond outflow. Our results corroborate that street runoff carries significant toxic potential and needs improved treatment prior to release into the aquatic environment.

3.12.P-Th102 Evaluation of Contaminants Reduction by Advanced Waste Water Treatment Via Effect-Based Methods

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In the European Union's Water Framework Directive the contamination of water with active pharmaceutical ingredient (API) residues is considered as an emerging environmental concern. Municipal waste water treatment plants (WWTPs) are relevant point sources of APIs as they treat the wastewater from public households, hospitals, and industry of the connected catchment area. APIs have beneficial effects on human and animal health, but in the environment their effects are a global concern. The environmental fate of most pharmaceuticals and their effects on biota are poorly known. Conventional "state-of-the-art" WWTPs

can only remove APIs that are either easily biodegradable and/or absorbable to activated sludge, whereas others can pass the treatment process with no or only minor reductions. Therefore, reduction of a broad range of APIs can only be achieved by using targeted advanced waste water treatment techniques, such as ozonation. The EU interreg project 'Clear Waters from Pharmaceuticals' (CWPharma) focused on filling the gaps of knowledge, e.g., regarding environmental concentrations and ecotoxicological effects of APIs. The project studied the impact of ozonation and post-treatment on ecotoxicological endpoints and established a guideline for advanced API removal. During an implementation phase (CWPharma2) technical solutions such as ozonation and different post treatments have been further evaluated at five different WWTPs in Denmark, Sweden and Germany. The ecotoxicological tests identified in CWPharma regarding effects of estrogenicity (YES test), mutagenicity (Ames fluctuation test) and bioluminescence inhibition (Microtox test) have been performed with waste water samples of secondary effluent before and after ozonation and with additional filters or biofilm reactors as post-treatments. The present contribution will give insights in the ecotoxicological results of test with enriched samples (SPE enrichment). The findings demonstrate that effect-based methods are valuable tools to directly indicate a contaminant reduction via (advanced) waste water treatment, e.g. for estrogenic compounds, and therefore, allow conclusions on the effectiveness of different API emission reduction measures.

3.12.P-Th103 Fate of Benzotriazoles During Anaerobic Digestion of Sewage Sludge

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In this study, lab-scale experiments were conducted to study the fate of benzotriazoles (BTRs) during anaerobic digestion of sewage sludge. These compounds are used in corrosion-inhibiting products, cooling fluids, de-icing fluids and dishwashing detergents and they are commonly found in Wastewater Treatment Plants treating domestic and industrial wastewater. A lab-scale CSTR anaerobic reactor was operated for a period of about six months at mesophilic conditions and at an HRT of 20 days using secondary sludge (from the Sewage Treatment Plant of Mytilene, Greece) as feed. The performance of the conventional anaerobic digestion was compared with that achieved in the presence of conductive material (magnetite) and when external low voltage (0.8V) was applied. In each experimental phase, the system was monitored for biogas/methane production and treatment efficiency (COD and VS removal as well as Volatile Fatty acids accumulation). When stable conditions were achieved in each experimental phase, a mixture of different BTRs was spiked in the reactor and samples were periodically taken to study the fate of target micropollutants during anaerobic digestion.

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3.12.P-Th104 Assessment of Watch List Chemical Occurrence In Surface WATER During a 4-Year Monitoring Programme

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Water quality is impacted by chemical compounds from a range of anthropogenic sources. Some of these chemicals are monitored because infirmation on their risk is well known. However, emerging chemicals of concern which do not have adequate data on occurrence in the environment are being added to the Watch List. The first full WL was established in Decision 2015/495/EU (2015) [1]. The original WL contained 10 suspected micropollutants or groups of micropollutants (also known as Contaminants of Emerging Concern (CECs)), totalling 17 individual chemicals. The criteria used for inclusion into the WL can be summarised into two key elements. An analytical approach involving solid phase extraction and liquid chromatography tandem mass spectrometry was developed for monitoring Watch List substances under the Water Framework Directive in Irish surface waters. This is the first time data for the full expanse of the Watch List has been generated in Ireland. Conditions from SPE extraction to analysis were optimized for recovery of a broad range of compounds at the ng L⁻¹ concentration level. Two LC-MS methods were used, in which estrogens were determined separately to the other analytes. LC-MS/MS methods for the analysis of the full suite of WL chemicals were developed and applied to 21 field samples taken from around Ireland over a four-year period. An extraction and analysis method was developed for the majority of analytes in the WL which achieved targeted LODs set by European standards, however future work is required in order to achieve targeted LODs for the estrogen group. Significant effects from the sample matrix were observed, particularly in the 2020 set of samples in which very high sample turbidity was present, indicating these compounds are highly sensitive to matrix interferences.

3.12.P-Th105 Microwave Regeneration of Granular Activated Carbon Saturated With PFAS

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To date, adsorption through granular activated carbon (GAC) is considered a well-established treatment for PFAS removal from contaminated water. However, the regeneration of PFAS-exhausted GACs is currently an important challenge for both water utilities and activated carbon manufacturers. Indeed, regeneration techniques such as chemical, biological and thermal are energy-consuming and/or partial effective because of the unique properties of PFAS. Microwave (MW) irradiation is a promising alternative to conventional thermal heating that has been proposed as an innovative technique for regenerating GACs saturated with dyes and pharmaceutical compounds. This study investigates the application of an innovative regeneration technique based on MW irradiation for PFAS-saturated GACs. Specifically, the MW regeneration of a commercially available GAC saturated with perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), selected as representative long-chain PFAS, was tested

through MW irradiation. The effectiveness of MW irradiation was assessed in terms of adsorption capacity recovery, while the effect of MW irradiation on GACs was examined by assessing the carbon weight loss percentages. The multiple-regeneration performance was also evaluated by conducting five successive adsorption/regeneration cycles. Moreover, the changes in BET surface area, pore volume, and pore size ascribed to several MW regeneration cycles were compared with virgin GAC properties. Textural properties of MW regenerated GAC did not noticeably differ from virgin ones. The obtained findings demonstrated that MW irradiation could effectively regenerate PFOS- and PFOA-saturated GACs. High temperatures eligible to desorb PFAS were reached in short irradiation times and the obtained values of adsorption capacity recovery jointly with a moderate weight loss encourage further investigations. Indeed, current investigations are focused on the regeneration of real field PFAS-saturated GACs in order to deeply explore the efficiency of MW regeneration in the co-presence of short-, long-chain PFAS and other water constituents.

3.12.P-Th106 Degradation and Ecotoxicity of Quaternary Ammonium Compounds (QACs) Subjected to Combined Vacuum UV and Uv-C Treatment

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Quaternary ammonium compounds (QACs) are active ingredients in a palette of commercially available disinfectants, sanitizers and biocides used across the globe because of their broad-spectrum antimicrobial properties. Application of QACs in Europe as pesticides, biocides and disinfectants is regulated by European Chemicals Agency (ECHA). Their widespread uses have resulted in frequent detection in many aquatic and terrestrial matrices including domestic wastewater, urban soils, surface waters and sediments. QACs have demonstrated efficacy in inactivation of more than 140 different pathogens, including SARS-CoV-2 virus and the use of these agents has increased concurrent with the emergence of the COVID-19 pandemic. However, increased QACs consumption is likely affecting the load of these compounds into the environment and investigation of environmental fate and effects on non-target organisms is in demand as well as technologies to mitigate environmental release. In this study, we examined the potential of combined Vacuum UV (185 nm) and UV-C (254 nm) irradiation (VUV/UVC) to degrade common QACs and decrease the ecotoxicity. Combined VUV/UVC irradiation has a potential to degrade QACs due to generation of reactive oxygen species (e.g., hydroxyl radicals) that interact with the target molecule. Our study showed that combined VUV/UVC irradiation facilitated rapid degradation of common QACs such as benzalkonium chloride, benzethonium chloride, didecyltrimethylammonium chloride, dodecyltrimethylammonium chloride, and hexadecyltrimethylammonium chloride. The efficiency of the degradation was affected by the initial QAC concentrations, the UV dose, and the water matrix. Toxicity of non-treated and UV-treated QACs was examined using a battery of test organisms from different trophic levels that included the luminescent bacterium *A. fischeri*, the gram-negative and gram-positive bacteria *E. coli* and *E. faecalis*, the freshwater microalga *R. subcapitata*, and the crustacean *D. magna*. The potential for trophic transfer of non-treated and UV-treated QACs in a simplified aquatic food web was also investigated. Test organisms from different trophic levels were included to better assess adverse effects of all bioactive compounds in UV treated samples including transformation products. The study suggests that combined VUV/UVC treatment of QACs resulted in significant photolysis coupled with noticeable decreases in toxicity to environmentally relevant organisms.

3.12.P-Th107 The Power of UV: Degradation and Toxicity Mitigation of Pesticides and Biocides by Combined Vacuum UV and Uv-C Treatment

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Pesticides and biocides are frequently detected as contaminants in groundwater and surface waters due to widespread societal use. Adverse effects have been reported for non-target organisms in a range of terrestrial and aquatic environments, and environmentally friendly methods for their mitigation are in demand. Advanced oxidation processes (AOPs) can potentially degrade organic contaminants in contaminated water and waste streams. UV based AOPs include Vacuum UV irradiation (VUV) that is considered a green technology that produces oxygen radicals including hydroxyl radical to degrade aquatic contaminants. However, the degradation efficiency and the toxicological profile of the process remain underinvestigated. This study investigated the potential of combined VUV (185 nm) and UVC (254 nm) irradiation (VUV/UVC) to degrade widely used herbicides, fungicides, and quaternary ammonium compounds (QACs) to decrease their toxicity. The study included the herbicide glyphosate and the transformation product AMPA, the triazole fungicide tebuconazole and the transformation product 1,2,4-triazole as well as common QACs. Glyphosate is the most widely used active ingredient in non-selective broad-spectrum herbicides, tebuconazole is an active ingredient in popular triazole fungicides used to control fungal diseases whereas QACs are used globally due to their broad-spectrum antimicrobial properties. Degradation kinetics, degradation mechanisms, and toxicity mitigation were investigated using different photoreactors. The results showed that combined VUV/UVC irradiation facilitated rapid degradation of common pesticides and biocides at the µg/L to mg/L level in different water matrices. The toxicity of non-treated and UV-treated pesticides and biocides was examined using a battery of test organisms with gram-negative and gram-positive bacteria (*A. fischeri*, *E. coli*, *E. faecalis* and *B. subtilis*), luminescent yeast (*S. cerevisiae* BLYR), filamentous fungi (*F. graminearum*), microalga (*R. subcapitata*), and crustaceans (*D. magna*). The test battery included organisms from different trophic levels to better assess adverse effects of all bioactive compounds in UV treated samples including transformation products. The study suggests that combined VUV/UVC treatment resulted in significant photolysis and noticeable decreases in aquatic toxicity of common pesticides and biocides due to mineralization of the parent compound or formation of less toxic transformation products.

3.12.P-Th108 Safeguarding Antibiotics Emission Into Aquatic Systems by Nature-Based Solutions

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Concerns on the occurrence of antibiotics, antimicrobial resistant microorganisms and microbial pathogens in aquatic ecosystems are increasing. So far, there is still little information and evidence about the capability of nature-based solutions (NBS) to reduce their impact on water quality. "Nature-based solutions to reduce antibiotics, pathogens and antimicrobial resistance in aquatic ecosystems" (NATURE) is a recently funded ERA-NET Cofund Aquatic Pollutants project with the aim of providing measures and technologies to reduce the impact of antibiotics on water quality. NBS are defined as "living solutions" inspired by nature to address various societal challenges in a resource-efficient and adaptable manner while delivering simultaneous economic, social, and environmental benefits. NATURE proposes to provide a holistic assessment of NBS as a management option of antibiotics from inland to coastal areas. Among the different specific objectives of the NATURE, we aim to evaluate and optimize the performance of secondary wastewater treatment by NBS. Aarhus University and Kilian Water ApS (Denmark) will work together to investigate the treatment of antibiotic residues by three different wetland designs: i) the most innovative hybrid bioelectrochemical wetland that will be benchmarked against ii) an aerated constructed wetland, and iii) a vertical flow constructed wetland. We will study similar size (approx. 125 PE sized beds) systems treating domestic wastewater implemented in Denmark. Systems will be monitored for 1 year by quantifying target model antibiotics and respective transformation products together with classical water quality parameters. In the second year of the project, operational conditions will be manipulated and the effects on treatment performance studied. Antibiotic resistance and pathogens will be further studied by other project partners. At SETAC Europe we will present the first monitoring results of antibiotic concentrations and treatment efficiency in the Danish pilot systems.

3.12.P-Th109 Ecotoxicological Characterization of Reverse Osmosis Concentrates and Antiscalants As Anti-Fouling Additives

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Modern techniques are steadily applied and optimized to ensure a high quality of drinking water. Therefore, purification processes, like membrane-filter technologies, including nanofiltration (NF) or reverse osmosis (RO), are used to meet these high-quality requirements. A concentrate is produced within the purification process, which is discharged directly or indirectly into surface waters. Additives, called antiscalants, that ensure a long membrane lifetime, and a mixture of anthropogenic trace substances are present in high concentrations in the concentrates. Antiscalants are mainly based on phosphonates- or polyacrylates that increase the solubility limit of free ions in the concentrate by complexation, reducing inorganic scaling on the membrane. However, they have a low biodegradability in the environment and can promote the migration of trace metals. Additionally, phosphate-based antiscalants contribute to the phosphorization of water and, thus to eutrophication. Therefore, in the *KonTriSol* project (funded by the Federal Ministry of Education and Research), different antiscalants (active ingredient and technical products) are ecotoxicologically evaluated. Additionally, selected concentrates from drinking water treatment plants are examined regarding their ecotoxicological potential. For this purpose, a bioassay battery is used to investigate acute (*Daphnia* immobilization test with *Daphnia magna*, algae growth inhibition test with *Raphidochelis subcapitata*, fish embryo acute toxicity test with *Danio rerio* and additional endpoints on mode of action, e.g. behavior alterations in light-dark transition test) and mechanism-specific toxicity (like PFAS-Calux on thyroid disruption). Subsequently, the investigated effects can be compared with those of the previously tested antiscalant and technical products. The first results obtained with an RO concentrate exhibited only toxicity to algae. However, the inhibitory effect of the RO concentrate on algae growth might be aside from possible inherent toxicity, caused by the nutrient complexing properties of Antiscalants, which will be addressed in more detail. The use of effect-based methods, consisting of mechanistic and whole organism-based tests, can provide a comprehensive overview of the ecotoxicological potential of the substances contained in the concentrates (including the respective antiscalant agents). The mechanism-specific tests can thereby contribute to identifying specific toxic substance groups.

3.13 Pelagic and Benthic Harmful algal blooms (HABs): The detection, fate, effects, monitoring, and management of blooms (Poster)

3.13.P-Mo193 Alterations on mRNA Expression of Interleukin Genes in Thymus and Spleen of Rats Orally Exposed to Cylindrospermopsin

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Cylindrospermopsin (CYN) is an ubiquitous cyanotoxin with increasing incidence worldwide. Humans can be exposed to CYN mainly by oral route through the ingestion of water or food contaminated with the toxin. CYN has been classified as a cytotoxin but it targets mainly the liver but also other organs. Among its toxic effects its immunotoxicity is scarcely investigated. The aim of this work was to assess the influence of CYN (18.75; 37.5 and 75 µg/kg/day) on the mRNA expression of selected interleukin (IL) genes (*IL-1b*, *IL-2*, *IL-6*, *TNF*, *IFN-?*) in thymus and spleen of male and female rats orally exposed to the toxin for 28 days. The gene expression was evaluated by Quantitative Real-time Polymerase Chain Reaction (qPCR). Results obtained showed that CYN

produced immunomodulation mainly in thymus of rats exposed to the toxin. In the thymus, immunomodulatory effects were observed after exposure to 75 µg CYN/kg/day in most of the genes, regardless of the sex. Moreover, up-regulation of *IL-2*, *IL-6*, *TNF* and *IFN-γ* were shown in females at the lowest dose of 18.75 µg CYN/kg/day. However, in the spleen only *IL-1b* and *IL-2* (males), and *TNF* and *IFN-γ* (females) were modified after exposure to the toxin. These outcomes highlight that CYN induces changes in the mRNA expression of IL genes in rats orally exposed to the toxin. Further studies are needed to obtain a deepest view of the molecular mechanisms involved in CYN immunotoxicity. Acknowledgement: The authors wish to thank the Spanish Ministerio de Ciencia e Innovación (PID2019-104890RB-I00 MICIN/ AEI/10.13039/ 501100011033) for the financial support. Leticia Diez-Quijada thanks the VI PPIT-US for the granting of a Postdoctoral Bridges Aid and its funding. Antonio Casas-Rodríguez acknowledges the Spanish Ministerio de Ciencia e Innovación for the predoctoral grant awarded (PRE2020-094412).

3.13.P-Mo194 Toxic Effects of Crude Extracts of the Phytoplankton Biomass Collected in Valle DE Bravo, Mexico on Early Life Stages of Danio rerio

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The Valle de Bravo reservoir is one of the main contributors to the Cutzamala System, that supplies drinking water to the Metropolitan Zone of the Valley of Mexico. This reservoir has been impacted by domestic, agricultural and tourism activities, causing deterioration in water quality and promoting eutrophication and the development of Harmful Algal Blooms (HABs), which are noxious to aquatic biota and humans. In this study the effect of crude phytoplankton biomass extracts obtained from the reservoir was assessed on the early life stages of *Danio rerio*. Biomass samples were collected from six locations in Valle de Bravo reservoir during March to December 2019. Crude extracts were obtained by cell rupture and bioassays were performed on recently fertilized *D. rerio* eggs, testing concentrations of 0.01, 0.1, 1, 1, 10 and 100 mg/L (dry weight). Cyanotoxins and accessory pigments concentrations were quantified. The results indicate that the crude extracts produced lethal effects such as coagulation and embryo mortality, as well as sublethal effects, such as absence of pigmentation, yolk sac oedema, curved tail, and teratogenic effects. The highest concentration of cyanotoxins was 2.16 µg/L determined in June, while the concentration of pigments indicates the highest levels in the months of April, August and September. In conclusion, the presence and frequency of the observed effects were related to the concentration of microcystins and other potentially toxic secondary metabolites within the reservoir.

3.13.P-Mo195 Development of Reproducible Culture Methods to Produce Cyanotoxins to Be Used to Generate Acute and Chronic Endpoints for Freshwater Organisms

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There is a lack of information to estimate safe exposure levels for aquatic life to natural toxins produced by cyanobacteria. The uncertainty in concentrations and purity of standards for cyanotoxins, and their high cost, challenge their use for acute and chronic toxicity tests. An alternative approach was tested, using cultures of cyanobacteria and algae to generate toxins. In this study, we have established laboratory cultures of toxin producers *Microcystis aeruginosa* lab cultured environmental strain (microcystin), *Aphanizomenon flos-aquae* PCC7905 (cylindrospermopsin), and *Dolichospermum circinale* CS-337 (saxitoxin) as well as non-toxin strain of *A. flos-aquae*. Early tests were conducted with microcystins obtained by removing cells from culture media and lysing them. Microcystin concentrations varied if cell density and time since inoculation (~150 days) of culture were tracked, with 1.75 X 10⁶ cells/mL resulting in a microcystin concentration of 885 µg/L, while 4.16 X 10⁶ cells/mL yielded 37 µg/L. Acute tests conducted with *Ceriodaphnia dubia*, *Neocloeon triangulifer*, *Hyalella azteca*, and larval *Pimephales promelas* using microcystins, did not cause any lethality different from the control at concentrations as high as 74 µg/L. However, the non-toxin-producing strain of *A. flos-aquae* caused mortality greater than the controls to *N. triangulifer*. For chronic tests IC25 for total microcystins for *C. dubia*, *P. promelas*, *H. azteca*, *N. triangulifer* were 8.9, 74.0, 408.9, and 10.1 µg/L, respectively. Due to the variability in getting consistent intracellular toxin levels, an approach was taken to harvest *M. aeruginosa* cultures during stationary phase to quantify microcystin concentrations by ELISA. Reproducible toxin concentrations (varied < 20%) were attained in both 500-mL and 1000-mL cultures, however, lysed cell extracts were too low to use for testing. These cultures were maintained at 25 +/- 1°C under a 16:8 h light/dark cycle at 15± 1 µmol m⁻² s⁻¹. A culture of this same species under lower temperature and illumination resulted in higher cell lysate concentrations after 6 to 12 months. These culture conditions were repeated, and the lysate concentrations were tested. Results of the new culture methods used for *M. aeruginosa* and acute and chronic toxicity results will be presented.

3.13.P-Mo196 Cylindrospermopsin, Anatoxin-a and Their Homologs in the Southern Czech Republic - Taxonomical, Analytical, and MOLECULAR Approaches

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Water bloom-forming cyanobacteria have severe impacts on freshwater quality including production of cyanobacterial toxins.

Despite of extensive research There is lack of information on the occurrence of neuro- and cyto-toxic toxins and their structural analogs as well as cyanobacterial taxa producing these toxins¹. The present study aimed to map levels of anatoxin-a (ATX), cylindrospermopsin (CYN) and their homologs along with the occurrence of *anaC* and *cyrJ* genes in laboratory strains and in water blooms from the Czech Republic providing thus the first integrative study combining analytical and molecular-biology approaches. Phytoplankton samples for taxonomical, toxin and molecular biology analyses were collected from the surface water in 16 localities in the southern regions of the Czech Republic. Freeze-dried biomass samples were extracted by acidified 75% v/v methanol and analysed by LC-MS for the content of microcystins, ATX and its homologs (HATX, DATX, DHATX), and CYN and its homolog 7-deoxyCYN. Qualitative PCR analysis of *cyrJ* and *anaC* genes was performed using DNA extracted from cyanobacterial biomass samples. **1. 2. 3.** The presence of CYN, ATX and their homologs HATX and 7-deoxyCYN was confirmed in phytoplankton biomass from 6 out of 16 pre-selected reservoirs. The presence of CYN and 7-deoxyCYN (up to 4.3 mg/g biomass d.w.; Píseňský pond) correlated with the presence of *cyrJ* gene. The corresponding calculated dissolved concentrations were 1.8 and 1.2 mg/L for CYN and 7-deoxyCYN, respectively. Interestingly, the biomass that contained both CYN and *cyrJ* gene was dominated by invasive cyanobacteria *Raphidiopsis raciborskii* (*Cylindrospermopsis raciborskii*) and *Cuspidothrix issatschenkoi*, and these cyanobacterial species might be newly described CYN producers in Europe. We further provided some of the first data on the occurrence of ATX and its congeners in the Czech Republic. ATX and HATX were detected in 4 natural biomass samples. The max concentration was 2.8 mg/g biomass d.w. for ATX and HATX. The calculated max dissolved concentrations were 365 ng/L for ATX (Site 13) and 12 ng/L for HATX (Site 8). Co-occurrence of *anaC* genes and neurotoxins (ATX, HATX) was not found in any sample. Interestingly, traces of ATX without confirmation of the *anaC* gene were observed in 2015, while contrary results (*anaC* gene but no ATX) were previously observed in 2013 in one reservoir (Stankov). The relevance of ATX congeners was further supported by analyses of laboratory strains, where levels of ATX congeners were about 10-times higher than those of the ATX itself.

3.13.P-Mo197 Trace Level Analysis of Odorants in Drinking Water Using High-Capacity-Sorptive Extraction

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The presence of volatile organic compounds (VOCs) in drinking water even at trace-level can have a significant effect on consumer experience. The naturally occurring compounds (*e.g.*, geosmin) have particularly strong odours and are often the source of complaints to water companies, while other emerging odorants (*e.g.* isopropyl methoxy-pyrazine) are also of concern. International methods (*e.g.* ISO 17943 and the Chinese regulation GB 57493) define low detection limits (*e.g.* 1 ppt for TCA) for these compounds. This study focuses on five malodorous compounds that are regulated across international regulations, which have been regularly associated with pelagic and benthic harmful algal blooms (HABs). Reliable detection of these compounds is therefore important, but is challenged by their low odour thresholds, which are typically at the low ng/L level. By using multi-phase (DVB/CWR/PDMS) high-capacity sorptive extraction (HiSor) combined with further analyte preconcentration using sub-ambient trapping, low levels of odorants can be analysed while maintaining peak shape, for increased signal-to-noise response, excellent linearity, low MDLs and highly reproducible results. The low detection limits enable water distribution facilities to detect malodorous compounds before consumers can. With the more robust, inert stainless-steel high capacity sorptive extraction probes similar results are achieved. The addition of multiple phases (DVB and CWR) to the HiSor probes enables extraction of more polar compounds, that may also indicate the presence of HABs. In this study, we demonstrate the fully automated sampling and detection of odorants at ng/L levels in water using headspace HiSor technique based on the principle of sorptive extraction and how trap-based preconcentration benefits the sensitivity, robustness and increases sample throughput, all of which is compliant with GB 57493 and ISO 17943.

3.14 Persistent, Mobile and Toxic (PMT/vPvM) substances: New Perspectives and Developments in their Assessment, Management and Regulation

3.14.T-01 Sfc-Ms Based Smart Screening of Groundwater for PMT/VPvM Compounds

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Persistent and mobile (PM) chemicals (also called PMOCs or in terms of regulations PMT/vPvM) infiltrate aquifers much faster than common compounds. Neither PMT/vPvM substances nor the compartment groundwater is considered by the regulation of REACH chemicals explicitly. This regulative gap originates from a knowledge gap: Especially the very mobile substances cannot be detected by common analytical methods like reversed phase chromatography coupled the mass spectrometry (RP-MS). Hence, the occurrence of PMT/vPvM compounds in groundwater is widely unknown. However, more than 55 % of EU drinking water is gained from groundwater. To protect the pollution of groundwater, the mobility and persistency of PMT/vPvM chemicals need to be accessed. The occurrence of PMT/vPvM chemicals in groundwater helps to estimate the PM characteristics necessary, to infiltrate this compartment. Hence, findings in the field help, how to narrow down the characteristics of PMT/vPvM substances, to put groundwater into risk. A screen smart study was conducted, which is crucial and urgently needed to reduce the analytical and knowledge gap by identifying hitherto unknown PMT/vPvM contaminants in groundwater. A new large volume generic enrichment method based on freeze-drying was developed to overcome the low injection volumes, coherently linked with the analysis of the extremely polar ones in the group of PMT/vPvM substances. In combination with supercritical fluid chromatography mass spectrometry (SFC-HR-MS) unknown PMT/vPvM chemicals were identified in groundwater. Total 15

compounds were identified, most of which are predicted to be negatively charged and with a negative logD in the relevant environment. Some of the identified compounds are toxic to reproduction, endocrine disruptors, or potentially carcinogenic. This study represents one important first step to identify the occurrence of PM compounds and thereby underlining the necessity of further extensive monitoring of these substances in groundwater, to access the mobility and persistence characteristics in field.

3.14.T-02 Persistence Assessment in Water-Sediment Systems

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In surface waters, aerobic biotransformation is considered the most dominant removal pathway for many contaminants. Therefore, international regulatory frameworks require the assessment of a substance's potential for biotransformation in laboratory studies following OECD 308/ 309 guidelines prior to its marketing authorization. To date, several shortcomings of OECD 308/ 309 studies have been elucidated; generally, study outcomes are often highly variable and not reproducible. A thorough understanding of relevant transformation processes and system-specific differences is still missing. Here, we performed laboratory studies with >40 compounds (i) as suspension tests containing sediment concentrations within and beyond standards defined in the OECD 309 guideline, and (ii) in a stagnant system in which a sediment layer is covered by a water column, i.e., OECD 308-type experiments. Outcomes of suspensions tests were highly variable and displayed striking inter-replicate and inter-study variabilities resulting in lag phases and chemical half-lives that differed by up to two order of magnitude for the same compound. When analyzing data of standard and modified OECD 308-type experiments, which employed sediment-water ratios of 1:3 and 1:10 (v/v), respectively, inter-replicate variabilities were strongly reduced. However, during standard OECD 308 studies, large fractions of compound mass tended to accumulate in the sediment layer and were not available for biotransformation, resulting in half-lives exceeding the typical duration of standard OECD 308 studies. Furthermore, with phase transfer processes dominating compound removal from the water column, outcomes of standard OECD 308 studies were found to be strongly dependent on properties of the employed sediment. Reduced sediment content in modified OECD 308-type studies compared to the standard OECD 308 setup allowed to shift observed processes from sorption to (bio)transformation. Compared to standard OECD 308/ 309 studies, the modified OECD 308-type system showed little inter-replicate and inter-study variability and improved observability of biotransformation for the majority of the investigated compounds. Overall, modified OECD 308-type studies thus allow for a better interpretability of a compound's potential for aerobic biotransformation.

3.14.T-03 Leachability As a Process-Based Method to Determine the Mobility of Chemicals in a PMT / vPvM Framework

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PMT and vPvM are envisaged as new hazard classifications for CLP to indicate chemicals with potential to reach drinking water sources by passing natural barriers like soil or riverbank sediments. As new criterion for mobility the Koc-value is proposed to be used to decide on mobile (M) or very mobile (vM) classification. However, in the ECETOC technical report No 139, evidence is provided that the Koc alone is a poor metric for the potential of chemicals to reach drinking water sources since this is mainly the result of the combination of both, sorption and degradation. The FOCUS framework for leaching of PPP through soil to groundwater which is a regularly updated approach that has been used for many years in a regulatory context is proposed as alternative. Most relevant processes such as degradation and sorption in soil as well as influences of climate, soil types and plants under representative European conditions are considered. Since PMT hazard classification for CLP must be based on substance inherent properties and should be independent of exposure leachability is defined as percentage leached mass related to the mass at the soil surface. Simulations were carried out with FOCUS-PELMO for all groundwater scenarios. The reference temperature for DT50 was set to 12 °C (ECHA guidance). Winter cereals was used and substance application was simulated at each month to consider the variation of the weather. The 80th percentile annual mass flux was related to the annual applied mass yielding the leachability in percentage. The average leachability of all scenarios was finally used as metric. Results of a sensitivity analysis show that leachability decreases rapidly with increasing Koc values and decreasing DT50 value. Substances that rapidly degrade in soil and sediment do not have the potential to reach drinking water sources despite low sorption although they might be classified as P or vP due to their persistency in other media like natural surface water (e.g. OECD 309). The use of the leachability approach for the M and vM assessment within PMT represents a much higher degree of realism than using a simple Koc-value. Due to the conservative assumption of only one meter transport distance and since transport times in the scenario for leachability are in the same order of magnitude as in typical bank filtration situations the results are in principle also transferable to such a drinking water protection scenario.

3.14.T-04 A Framework to Address Challenging Substance Properties and Improve the Use of Weight-Of-Evidence in Persistence Assessments - Outcome of the CEFIC-LRI ECO52 Project

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Chemical persistence plays a key role in the determination of environmental exposure, making it an important component in risk assessment and regulation. However, existing frameworks under which persistence is assessed have shown some challenges. Chemical degradation rates are variable and dependent upon a number of factors including environmental conditions, which is difficult to address in a weight of evidence approach. In addition, many substances are problematic, or fall outside the

applicability domain of existing frameworks due to their specific characteristics. These include substances with certain phys-chem properties that render them difficult to test, substances of unknown composition, complex reaction products or biological materials (UVCBs), and polymeric substances. Finally, evaluating degradation half-lives using a compartment-by-compartment approach is overly simplistic, and neglects dynamic multimedia exchanges and degradation processes that may have an important bearing on the overall persistence of a substance in the environment. The CEFIC-LRI ECO52 project aims to address these issues by improving available guidance for persistence assessment of substances, including those considered problematic to evaluate under the current frameworks. This presentation will discuss the overall outcome of this CEFIC-LRI project, and the assessment framework for persistence that has been developed to deliver three important elements: 1) Improved guidance on weight of evidence determination, incorporating bioavailability as well as critical processes and compartments of concern, leading to more robust and consistent decision-making in line with persistence protection goals. 2) Provisions for the assessment of substances currently problematic or outside the applicability domain of persistence frameworks, namely difficult test substances, UVCBs and polymers (including microplastics). 3) A workable methodology for incorporation of multimedia fate modelling and ‘overall persistence’ consideration into the persistence assessment framework. It is anticipated that these developments will lead to improvements to the transparency, consistency and robustness of persistence assessments and their applicability to a range of substance types in the future.

3.14.T-05 Trifluoroacetate Leaching Potential From Fluorinated Herbicides - a Balancing and FOCUS Modelling

Approach

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Trifluoroacetate (TFA) is a very mobile, highly soluble and very persistent substance which originates from various chemicals containing a C-CF₃-group. One major source are plant protection products (PPP), which underlie aerobic degradation in soil and form TFA that enters groundwater via direct leaching. Accordingly, TFA was detected in many water bodies in elevated concentrations and is present in drinking water as well. During the approval process of active substances in PPP on EU level, the formation of TFA is not systematically investigated. Moreover, the potential of a precursor to form TFA during aerobic degradation studies may remain unrevealed as a specific analytical method is required. If TFA formation is then overlooked during the approval process, TFA is not included into groundwater risk assessment. The present work aims at estimating TFA entries into groundwater despite the lack of experimental data. We investigate whether TFA formation is an issue when using PPP and whether it may need more attention during the EU approval process of active substances. Here, we present the results for eleven herbicidal substances. Firstly, the TFA formation potential of the precursors was balanced and refined by an inclusion of mean production areas of the respective crops in the EU. Secondly, groundwater concentrations were modelled with FOCUS PEARL based on the peer reviewed endpoints published by the European Food Safety Authority (EFSA). Metabolism schemes were used as specified for groundwater modelling in the EFSA conclusions/renewal assessment reports modified for TFA formation and excluding those metabolites that do not contain a C-CF₃-group. Balancing of TFA emission potential shows that TFA might be emitted in high amounts and on a large area within the EU. The results of groundwater modelling indicate a medium to high leaching potential for TFA from all herbicides and crops considered. Hence, TFA formation and leaching to groundwater requires more attention in the approval of active substances used in PPP. TFA formation should be investigated in aerobic degradation studies for all substances containing a C-CF₃-group as standard data requirement and included into groundwater risk assessment to obtain a more realistic insight into TFA entries. Furthermore, these results may be used for groundwater monitoring concepts and in support of local minimization strategies in vulnerable areas.

3.14 Persistent, Mobile and Toxic (PMT/vPvM) substances: New Perspectives and Developments in their Assessment, Management and Regulation (Virtual Only)

3.14.V-01 Are the Persistence and Mobility of Chemicals Linked to Their Potential to Contaminate Drinking WATER?

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Persistent (P) and Mobile (M) organic chemicals are considered emerging threats to the environment and drinking water because they can be transported long distances, penetrate natural and artificial barriers, and resist removal by traditional water treatment procedures. Bright-line P and M hazard criteria have been proposed as proxies for evaluating the potential for chemicals to reach and contaminate drinking water. While these screening criteria are simple and straightforward, it remains unclear whether, and to what extent, they capture the complex reality of chemical transport in the environment and hence adequately represent a chemical's potential for contaminating drinking water. In this work, we compare and contrast proposed P and M hazard criteria with concentrations in drinking water predicted by a fate and transport model, based on the data of 112,000+ discrete organic chemicals registered in chemical inventories. We evaluate whether chemicals with the greatest potential to contaminate drinking water are those scored high in both the P and M assessments. We find that while chemicals scored high in P and M indicators tend to possess a higher potential to contaminate drinking water, the difference in the potential between high and low P- and M-level chemicals is not statistically significant. Screening chemicals based on P and M indicators alone may overestimate the occurrence of certain chemicals in drinking water and hence causes “false positive” cases. While there is some value in identifying these types of chemicals, we also demonstrate it is also possible and prudent to characterize these chemicals in terms of exposure and potential risk. This work addresses the academic and regulatory need for better understanding the processes and properties relating

to drinking water contamination and for developing and evaluating scientifically defensible methods and criteria to identify and potentially regulate chemicals of concern for the safe and sustainable use of chemicals in commerce.

3.14.V-02 Interpolation and Extrapolation of Polyparameter Linear Free Energy Relationships (PP-LFERs) to Predict Partition Coefficients

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Polyparameter linear free energy relationships (PP-LFERs) predict equilibrium partition coefficients (K) of diverse neutral organic compounds and thus are useful to evaluate the mobility of chemicals. PP-LFERs describe the free-energy contributions of all intermolecular interactions relevant to solute solvation using five compound descriptors, undergo training with experimental data through the least-square multiple linear regression method, and generally have a broad range of applicability. Like any other empirical model, the prediction accuracy of a PP-LFER depends on the quality of calibration, and extrapolations outside the model calibration domain are expected to be less accurate than interpolations. Moreover, a long-range extrapolation should more likely generate a large error than a short-range extrapolation. However, quantitative evaluation of interpolation and extrapolation of PP-LFERs has seldom been performed. In this study, the applicability domain (AD) of PP-LFERs is evaluated by calculation of the leverage (h_j), a measure of distance from the calibration domain as defined by the five compound descriptors. Simulations with experimental data illustrate that the root mean squared error of predictions indeed increases with h_j . Moreover, extreme prediction outliers occur more frequently when h_j exceeds the common threshold of $3h_{\text{mean}}$, where h_{mean} is the mean of leverages of all training compounds. Nevertheless, well-calibrated PP-LFERs with many (e.g., 100) and diverse training data are surprisingly robust against extrapolation. Per and polyfluorinated alkyl substances (PFASs) with large F/C ratios and organosilicon compounds (OSCs) have characteristic interaction properties and thus are always outside the domain of calibration if none of them are included in the training set. Thus, these compounds must be extrapolated by typical PP-LFERs and be prone to elevated prediction errors. Published PP-LFERs are evaluated with 25 AD probe chemicals in terms of the domain of calibration, which shows that existing PP-LFERs are differently biased; highly polar chemicals and/or highly hydrophobic chemicals are often out of the calibration domain in addition to PFASs and OSCs.

3.14.V-03 Online Analysis of Gas and Particle Phase Trifluoroacetic Acid

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Trifluoroacetic acid (TFA) is an ultrashort chain perfluoroalkyl carboxylic acid (PFCA) containing two carbons that is expected to increase in the environment following the Montreal Protocol-mandated chlorofluorocarbon (CFC) replacements. TFA is highly polar, stable in the environment, and highly soluble in water making them very persistent and very mobile (vPvM). Current atmospheric sampling methods for short-chain PFCAs mainly use offline sampling techniques, such as measuring gaseous TFA with annular denuders and particulate TFA with quartz filters on collection timescales of several hours to days. The atmospheric processes (e.g. gas-particle partitioning) that drive production and fate of ultrashort-chain PFCAs occur on faster timescales. As a result, these slow measurements offer limited insight into these processes. Faster analyses can give extremely valuable information on the temporal trends and concentrations of analytes. There is currently a gap in research of simultaneous semi-continuous analysis of gaseous and particulate TFA. This study collected gas and particle phases hourly in Toronto, ON Canada using an ambient ion monitor ion chromatograph mass spectrometer (AIM-IC-MS). The benefit of this is it can simultaneously analyze major inorganic and organic charged ions that assist in understanding the behavior of gas-particle partitioning. By coupling the AIM-IC to a mass spectrometer (AIM-IC-MS), we will demonstrate: i) the extended capability of the technique to ultra-trace chemical species, including TFA; and ii) the first in situ measurements of gas and particle TFA in the ambient atmosphere. TFA was present in almost all samples with higher amounts of gas phase TFA compared to the particle phase. Variation of TFA concentrations throughout a day can be seen with an average of total TFA at $2.4 \pm 0.9 \text{ ng m}^{-3}$ in November. This represents an increase in atmospheric TFA previously reported in the early 2000s.

3.14.V-04 PMT and vPvM Substances in Republic of Korea

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Persistent, mobile and toxic (PMT) substances and very persistent and very mobile (vPvM) substances have been received the deserved attention. The intrinsic properties of PMT and vPvM substances hamper consistent and reliable monitoring, control, and regulation of those chemicals. In this study, PMT and vPvM substances are categorized and prioritized to construct a new database of the Republic of Korea. The construction of PMT and vPvM substances database would be the first step of discussion on how to deal with PMT and vPvM substances. Herein, several indicators were considered including the consumption and use of substances, detection frequency in environment, detected concentration, and toxicity. Over 100 compounds were selected, many of which were overlapped with the existing list in EU commission. With the produced database, the analysis methods of PMT and vPvM substances in environment were proposed by using passive sampling approach.

3.14 Persistent, Mobile and Toxic (PMT/vPvM) substances: New Perspectives and Developments in their Assessment, Management and Regulation (Poster)

3.14.P-We110 Environmental Behaviour and Mobility of Heterocyclic PAHs - Hazard Classification Towards PMT and PBT Concepts at Screening Level

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Heterocyclic polyaromatic hydrocarbons (heterocyclic PAHs) are of particular interest for environmental and human impact assessment due to their environmental abundance, indication of persistence, environmental mobility and poor data availability in many aspects. Heterocyclic PAHs have frequently been detected in the environment along with homocyclic PAHs, often near petroleum-contaminated sites. More importantly, the detection of heterocyclic PAHs in areas not specifically impacted by coal or oil mining, or processing activities, suggests high atmospheric or geohydrological mobility of heterocyclic PAHs accompanied by lack of degradation or their formation from other primary contaminants. Nevertheless, little attention has been paid to heterocyclic PAHs so far in terms of environmental behavior or hazard assessment. To address this, we first experimentally determined the physicochemical properties of 18 two- to five-ring heterocyclic PAHs, namely octanol-water partition coefficient, water solubility, and organic carbon-water partition coefficient (K_{oc}). In view of the increasing pressure on (drinking) water resources caused by persistent and mobile organics, the heterocyclic PAHs under study were classified into mobility classes based on $\log K_{oc}$ values, which were obtained from simulation tests using HPLC. Out of 18 compounds 11 were identified as potentially persistent, seven as mobile or very mobile and 11 as bioaccumulative or very bioaccumulative based on screening-level QSAR models using newly obtained experimental physicochemical data.

3.14.P-We111 Using the Concept of Essential Use to Manage PMT/VPvM Substances in Consumer Products: A Case-Study for Cosmetics

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Due to their specific properties, substances that are mobile, persistent and toxic or very persistent and very mobile (PMT/vPvMs) can pose risks to water bodies. These substances are mainly unregulated, however, this is likely going to change in light of the publication of the Chemical Strategy for Sustainability in October 2020. Here, the European Commission expressed the aim to classify PMT/vPvMs as “substances of concern”. Furthermore, they announced their intention to extend the “essential-use concept” to risk management to ensure that consumer products do not contain harmful chemicals. The essential-use concept has previously been shown to be a useful tool to guide the phase-out of ozone-depleting substances under the Montreal Protocol, and has recently been suggested as a tool to guide the phase-out of other problematic groups of substances such as per- and polyfluoroalkyl substances. Extending the implementation of the essential-use concept to PMT/vPvMs requires a sufficient understanding of the current uses of substances and of the availability, suitability, and hazardous properties of alternatives. Currently, PMT/vPvMs are used in a diverse range of applications, including in cosmetic products. Studies indicated that cosmetic products contain a large number of chemicals with potential hazardous properties and that their use contributes to the occurrence of these chemicals in the environment. This emphasises the importance to manage hazardous substances, including PMT/vPvMs, in cosmetic products to both protect human and environmental health. Here, we aim to identify PMT/vPvMs in cosmetics products based on database searches. An initial screening of products based on a list of 20 PMT/vPvM showed that circa 6.4% of cosmetic products contain these substances. Haircare products (13.0%) and make-up (6.7%) are the two product groups with the highest share of PMT/vPvMs. Three of the most occurring substances -CI 16035 (colourant and hair dyeing agent), Benzophenone-4 (light stabilizer, UV absorber and UV filter) and Climbazole (anti-seborrheic and preservative)- were selected as case-study chemicals to explore the suitability of the concepts of essential-use and alternative assessment to manage and replace PMT/vPvMs in consumer products.

3.14.P-We112 Current Status of the Implementation of the PMT/VPvM Criteria Into CLP and REACH Regulation

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The European Green Deal (EGD) sets an ambitious zero pollution vision for 2030. The overall objective is to reduce the pollution of air, water and soil to levels that are not harmful to human health and the environment. In this context the European Commission published on October 14th 2020 its Chemicals Strategy for Sustainability Towards a Toxic-Free Environment (CSS). The CSS is one important pillar of the Zero-Pollution Ambition for a Toxic Free Environment. The European Union has one of the most comprehensive and ambitious legislations on chemicals globally. The Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (COM, 2006) and the Regulation on the Classification, Labelling and Packaging of hazardous substances (CLP) are the two horizontal legislations on chemicals in the European Union. The European Commission aims to finalise the legislative proposal for the revision of the REACH Regulation by the end of 2022. The European Commission also aims to strengthen the CLP regulation as cornerstones of EU chemicals legislation. The “One substance, one assessment” process will ensure simplification and greater transparency of chemical hazard assessment. In 2021 the process started to amend the CLP Regulation to introduce new hazard classes on endocrine disruptors, PBTs/vPvBs and PMTs/vPvMs and apply them across all legislation. On September 24th 2021 the Commission proposed new hazard classes and criteria in the CLP Regulation to fully address environmental toxicity, persistency, mobility and bioaccumulation. Initial discussion took place at ECHA’s PBT expert group. In an ad hoc meeting of CARACAL on September 30th 2021 the criteria for PBT/vPvB and PMT/vPvM have been discussed and subsequently commented in writing by the MS Cas, industry, NGOs and stakeholders. The underlying goal of adding new hazard classes related to PMT and vPvM is to protect natural resources that could be used for drinking purposes from chemical pollution. Here we will present the current status of the implementation of the PMT/vPvM criteria into CLP and REACH regulation since November 2021. We will present a summary and overview of the written comments and analyse the differences in the different sets of PMT/vPvM criteria. The new hazard classes PMT and vPvM will be applied to substances detected in raw water and drinking water.

3.14.P-We113 ZeroPM - Zero Pollution of Persistent, Mobile Substances

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Announcing the recently funded H2020 Project ZeroPM – Zero Pollution of Persistent, Mobile substances. ZeroPM will interlink and synergize three strategies to protect the environment and human health from persistent, mobile substances: Prevent, Prioritize and Remove. The project is rooted on EU's Chemicals Strategy for Sustainability towards a toxic free environment. To choose the PM substances for which this is most urgent, ZeroPM will Prioritize PM substances and substance groups through the development and application of robust screening and prioritization tools. These tools will identify all PM substances on the global chemical market, taking into consideration their production, use, presence in the circular economy, exposure, hazards and risks. To Remove, ZeroPM will focus on geographically impacted areas and prioritized groups of PM substances, and develop next generation remediation methods to remove PM substances from water resources, drinking water and sludge-derived products. *Acknowledgment: This project has received funding from the European Union's Horizon H2020 research and innovation program under grant agreement No 101036756*

3.14.P-We114 ZeroPM - Catalyzing the Market Transition Away From Persistent and Mobile Chemicals

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The ZeroPM workpackage “Market Transition” will work in close collaboration with industry (manufacturers and downstream users) to provide fit for purpose web-based tools to support companies at different stages of the market transition. Mainly three different tools to support this transition will be developed or updated: The SIN (Substitute it Now) List, the ChemSec Marketplace and a brand new “PFAS guide”. The SIN List is itself an “early warning” of chemicals that will be regulated and is often ahead of the much slower official regulatory process. As such it drives innovation in the chemical industry, having about 50 000 users per years from all over the globe. By using the results from the other work packages, an update of the SIN List with additional PM chemicals is planned for 2025. The ChemSec Marketplace gathers green chemical innovations in one place and allows chemical producers to advertise their products free of charge. This is a unique and much appreciated resource for different types of companies, as well as for regulators. Information on safer alternatives to PM substances, derived within the ZeroPM program will be published also as adds on the Marketplace website throughout the project. Finally, a brand new “PFAS guide” for companies supporting them in the identification of PFAS in their products or manufacturing processes will be established. Using the different available inventories on PFAS uses, many of which have been produced by project partners, an easily accessible interactive tool will be built in close collaboration with companies representing the target group. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 101036756

3.14.P-We115 Comparison of NER Formation in Soil (OECD 307) and Water-Sediment (OECD 308) Degradation Study Using ¹⁴C Labelled Phenanthrene

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Biodegradation data of chemicals are necessary for regulatory use and often generated using standard tests (e.g. soil degradation according to OECD 307 and water-sediment degradation according to OECD 308). In these tests many chemicals may form non-extractable residues (NER) which can be quantified only if the test is conducted with isotopic labelled chemicals. NER include by definition physically entrapped parent compound and/or transformation products (type I NER), which is potentially remobilizable and hazardous, non-remobilizable covalently bound (type II NER) and biogenic (type III NER) fractions. For characterization of these fractions, procedures such as silylation for release of type I or acidic hydrolysis for determination of type III NER have been proposed and tested in soil degradation studies. These procedures are also gradually being applied to sediment degradation studies, but so far experimental data are very rare. The aim of this work was to apply 1) the proposed procedure of type I NER determination to samples from a water/sediment study and 2) to compare the total NER and type I NER formation in soil and water-sediment degradation studies using the same test substance. The degradation of ¹⁴C-labelled Phenanthrene was tested in (i) two different soils according to OECD 307 and (ii) two different water/sediments systems according to OECD 308. The studies were run for a duration of 120d and 103d for soil and sediment, respectively. Soil samples were exhaustively extracted by solvent extraction followed by harsh extraction using accelerated solvent extraction (ASE). The sediment samples were extracted analogously, but ASE was only performed on a subset of samples, and deemed unnecessary due to low additional recoveries (< 5% of applied radioactivity (AR)). The remaining total NERs were quantified by combustion analysis and the extracted soil residues subjected to the silylation procedure. For the soil and sediment degradation studies, the total NER fraction after 28d

ranged from 35.5-39.3% AR and 35.3-41.5% AR respectively. Towards end of incubation, total NER decreased in both studies to 25.5-26.3 %AR for soil and 29.7-40.3%AR for sediments. Higher amount of parent could be identified in the silylation extracts of sediments (9.6 %AR parent in 16.9%AR released (28d)) compared to soil (~2.1 %AR in 14.7%AR released (14d)). The release of relevant amounts of parent from NER and the impact on persistency assessment will be discussed.

3.14.P-We116 Risk Indicators for PMT/vPvM Substances

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Substances that are very persistent in the environment and very mobile in the aquatic environment (vPvM); or, substances that are persistent in the environment, mobile in the aquatic environment and toxic (PMT) are of concern to the sources of our drinking water. A review of substances detected in drinking water and groundwater found that 43% of them are REACH registered (referring to the EU REACH Regulation (EC) No 1907/2006). Further, REACH registered substances were the most likely to be found at higher concentrations (above 0.1 µg/L). We developed guidelines to prospectively or retrospectively use the REACH registration process to identify PMT/vPvM substances. Special considerations for data uncertainty are presented via the implementation of a “traffic light” system. The guidance was applied to all 22400 REACH registered substances as of September 2019, and in addition 1308 substances that were reported as detected in various fresh water monitoring studies. The results of a PMT/vPvM hazard assessment for these substances will be presented. For a subset of these substances, a risk indicator assessment was developed that in addition of looking at the hazard of PMT/vPvM substances, also considers other factors associated with exposure and risk.

3.14.P-We117 ZeroPM - Policy Analysis, Development and Assessment

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The H2020 Project ZeroPM – Zero Pollution of Persistent, Mobile substances will include a work package focused on identifying policy changes to more effectively prevent PM substances from entering the environment. It will analyse policy objectives and targets formulated at European and international levels, including the EU Chemicals Strategy and the upcoming Zero Pollution Action Plan for Water, Soil and Air. Sources will include relevant environmental, sector, product and substance legislation, as well as stakeholder interviews and workshops. This evidence base will be used to spot opportunities and gaps in the existing policy/legal framework and to develop and assess policy options for minimizing emissions of PM substances. Work products will include policy briefs presenting key findings and recommendations for actions, along with roadmaps for meeting zero-pollution ambitions. *Acknowledgment: This project has received funding from the European Union's Horizon H2020 research and innovation program under grant agreement No 101036756.*

3.14.P-We118 ZeroPM - Removal of PM Substances

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In the H2020 Project ZeroPM – Zero Pollution of Persistent, Mobile substances, there will be a Work Package dealing with the removal of persistent and mobile (PM) substances.

Within this Work Package, geographically impacted areas and prioritized groups of PM substances like PFAS will be focused and developed next generation remediation methods to remove PM substances from water resources, drinking water and sludge-derived products will be investigated. Various aspects of PM substance exposure in drinking water, water resources and sludge will be investigated through three test sites in Germany and Greece. It will be demonstrated how and if legacy PM substance pollution can be remediated.

Specifically, monitoring methods and strategies will be initially developed and applied to facilitate the evaluation of the different water treatment methods. This will include both the adaption of existing technologies for on-site real time monitoring and innovative methods including passive sampling (PS), high-resolution analysis of PM substances and sum or group parameters to overcome shortcomings of existing analytical methods. For the treatment of contaminated groundwater, two advanced and innovative technical solutions for the PM substances will be tested. Concerning wastewater and sludge management, lab and pilot scale experiments will be conducted for PM substance contaminated sludge and wastewater in order to evaluate removal efficiency and to gain insight into degradation pathways, using a combination of new and improved biological and thermal treatment processes.

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3.14.P-We119 ZeroPM - Risk Assessment

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In the H2020 Project ZeroPM – Zero Pollution of Persistent, Mobile substances, there will be a Work Package dealing with the development and application of risk assessment methods targeted for chemicals identified as persistent and mobile (PM). Within the Risk Assessment Work Package there will be an emphasis towards the development of a multimedia fate model aimed at estimating environmental concentrations based on an evaluation of multi-pathway exposures of chemicals into the environment. The predicted environmental concentration will then be used to derive an external human exposure concentration to PM substances in drinking water. The estimated drinking water concentration will be used in combination with the development of a physiologically based pharmacokinetic (PBPK) model, which will translate in vitro effect concentrations to human relevant internal doses of PM substances and external exposure scenarios, and vice versa. To support the ability to relate exposure to an adverse effect concentration, a toolbox for PM hazard assessment will be developed using a suite of in vitro and in silico methods targeting the main toxicological endpoints of concern. Ultimately, risk will be derived with results communicated using a risk matrix approach that can be used for screening and prioritization of PM substances based on integration of exposure and hazard data (contributing to WP3 and single substance regulation). The risk matrix approach will thus be used both retrospectively, whereby the approach can be evaluated against known PM substances, but is anticipated to provide valuable prospective insight by enabling the screening and identification of bioactive contaminants of emerging concern with substance properties consistent with potential PM substances.

3.14.P-We120 Expanding the Biodegradation Database in enviPath to Predict Transformation Products and Half-Lives and in Soil and Sediment

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Computational models of microbial biodegradation processes are designed to predict biotransformation pathways and half-lives of organic pollutants, but they require big amounts of accessible, standardized biodegradation data, which are difficult to obtain. To this end, the online platform enviPath has been established in the past [1]. It contains biodegradation pathways and half-lives for chemicals in the environment, complemented by metadata on study conditions. An integrated pathway prediction engine proposes potential biotransformation products and pathways. These predictions are performed by biotransformation rules and guided by a machine learning algorithm trained on the experimental data in enviPath. In this work, we build upon existing resources in enviPath to accommodate data from additional environmental compartments. The first environment-specific dataset has been published for pesticide degradation in soil (OECD 307) [2]. Here, we expanded the enviPath framework to accommodate experimental data from water-sediment systems (OECD 308), and we imported study data for pesticides from public regulatory dossiers. In addition, we added biodegradation data for activated sludge (OECD 314b) to lay the foundation for read-across approaches from sludge to soil and sediment studies. Adding new data types for more chemicals creates new opportunities for half-life modeling, prediction of transformation products, and reading across different environments. The enviPath web server is publicly available, and accessible via an Application Programming Interface (API) to facilitate integration into custom modeling workflows. Hopefully, the expansion of enviPath will open new avenues for modeling persistence of organic pollutants in different environments by providing standardized, easily accessible biodegradation data obtained from regulatory-type standard simulation studies. [1] Wicker et al., *Nucleic Acids Res.*, p. gkv1229, Nov. 2015. [2] Latino et al., *Environ. Sci. Process. Impacts*, vol. 19, no. 3, pp. 449–464, Mar. 2017. Acknowledgment: PREMIER has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 875508. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA (<https://www.imi.europa.eu/>).

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3.14.P-We121 Modelling Biodegradation of Organic Chemicals - Is Initial Degradation Concentration an Insensitive Parameter?

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Persistence is a key criterion in chemical risk assessment, and high persistence of any substance is of major concern. In chemical regulation, persistence is assessed in environmental simulation tests according to OECD technical guidelines and often with ¹⁴C-radioactive-labeled compounds. Typically, significant amounts of non-extractable residues (NER) of the label remain in the matrix. Part of this NER, named biogenic NER or bioNER, is formed by microbial biomass and microbial residues, which is of no concern in the persistence assessment. The degradation kinetics as well as the formation of bioNER can be simulated using Michaelis-Menten kinetics (v_{max} and K_s) combined with calculated biomass growth derived from theoretical growth yields [1, 2, 3]. This approach allows to extract, by inverse modelling and model calibration, kinetic parameters for microbial growth and metabolism and also enables the prediction of the bioNER formation. Different descriptors have been used to describe the total biomass and the degrader biomass. However, in the present study we can show that the initial amount of degraders may be an insensitive parameter for the model simulations: initial degrader biomass does affect the time passing until the exponential growth phase of the microbial population sets in (i.e. the lag phase) but has little effect on the final distribution of the applied label.

Therefore, initial values can be selected which are typical for the type of experiment (e.g. soil, water, water and sediment) and the inoculum. Once the growth and metabolism parameters have been determined by inverse calibration, the outcome is transferable to other compartments, with other initial conditions and concentrations, as well as to real environments. Inverse model simulations will allow to re-evaluate existing or future OECD test results without the necessity to measure bioNER or microbial population densities, to derive realistic, scientifically sound and transferable descriptions of degradation kinetics, and thus supports rational persistence assessment under consideration of NER formation without additional experimental effort. [1] Kästner et al. (2014). *Crit Rev Environ Sci Technol*, 44(19), 2107–2171. [2] Trapp et al. (2018). *Environ Sci Technol*, 52(2), 663–672. [3] Brock et al. (2019). *Environ Sci Technol*, 53(10), 5838–5847.

3.14.P-We122 Biodegradation of Heterocyclic Polyaromatic Hydrocarbons - Ready Biodegradability, Microbial Adaptation, Primary Biodegradation

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The past decades have witnessed ongoing progress in understanding the hazard potential of heterocyclic polyaromatic hydrocarbons (heterocyclic PAHs). Heterocyclic PAHs are often less hydrophobic and more water-soluble than their homocyclic equivalents, and are therefore more mobile and bioavailable in aqueous compartments. Large heterocyclic PAHs, although more polar than homocyclic equivalents, also have high affinity to soil organic matter and might be bioaccumulative. Smaller heterocyclic PAHs on the other hand are potentially mobile in soils and are not expected to bioaccumulate in biota. Due to their potentially hazardous properties, the elimination of heterocyclic PAHs from the environment is of paramount importance that mandates investigation. In this work, we tested the ready biodegradability of nine heterocyclic PAHs using the manometric respirometry method under aerobic conditions. None of the tested compounds proved to be readily biodegradable. The microbial community was then adapted to the test substances, but no improvement in mineralization was observed after a 60-day of adaptation period. Several compounds were found to be “inhibitory” to the microbiota adapted to test substances for 250 days, even when loaded at the lowest concentration (3 mg/L) viable for the manometric system. With the aim of seeking more feasible conditions for testing biodegradability of heterocyclic PAHs, a primary biodegradation test was designed following the course of biodegradation via GC/MS. After two successful primary biodegradation tests supplemented with abiotic controls, carbazole, dibenzofuran, benzo[a]acridine, and benzo[c]carbazole underwent primary biodegradation, with only carbazole (100%) and benzo[a]acridine (70%) showing high levels of biodegradation, which improved slightly in the second primary biodegradation test with longer adaptation period (120 days) compared to first run (75-day adaptation). Although compound removals were observed in primary biodegradation tests ($C_{\text{start}}=0.16$ mg/L), any oxygen consumption was not detected in the manometric system ($C_{\text{start}}=15$ mg/L), which could be due to the toxic effects of the compounds on microorganisms at high test concentrations. Yet, no evidence of biodegradation was detected for the rest of heterocyclic PAHs. These results suggest that the persistence potential of heterocyclic PAHs should not be underestimated, and urgent steps should be taken to closely monitor these compounds in the environment.

3.14.P-We123 Identification of Degradation Products - an ECHA Decision CASE Study for a Non-Readily but Inherently Biodegradable Substance

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The identification of degradation products is a standard information requirement of REACH Annex IX (?100tpa substances). We received an ECHA decision requesting this information for a substance that was not readily biodegradable but inherently biodegradable and not persistent based on positive results in enhanced biodegradation screening tests. ECHA considered that this information was needed in relation to the PBT/vPvB assessment. Two extended 301F ready biodegradability studies were included in the original dossier in which the pass level of 60% was achieved by day 42. Both studies met the conditions outlined in ECHA guidance (Chapter R.11) for when positive results from an enhanced screening may be used to conclude that a substance is not P/vP. However, it is noted that the ECHA guidance includes the proviso “together with other supporting information”. ECHA requested the identification of the degradation products by using an appropriate and suitable test method. The method had to be substance specific and, if analytically possible, the identification, stability, behaviour and molar quantity of the metabolites relative to the parent were to be evaluated. In this poster, we present how we successfully addressed this information requirement by conducting a new extended 301F test with the inclusion of substance specific analysis. The registered substance is an organic nitrile. It was hypothesised that the most probable primary metabolites are the corresponding amide and acid. Reference samples were synthesised from the parent substance. The concentrations of parent and target metabolites were quantitatively monitored by LC-MS throughout the test (e.g. day 0,1,4,7,14,21,28 and 60). At the end of the test, the parent substance was below the limit of detection, indicating complete primary biodegradation. Very low levels of the two metabolites were detected during the test. The target metabolite analysis coupled with the high BOD at the end of the test (73% day 60) indicates that any metabolites formed following primary degradation are transient and not persistent. This was supported by CATALOGIC predictions. With the submission of this new data, ECHA considered the request in the decision as met, and the information requirement fulfilled. Therefore, this case study demonstrates how OECD 301 studies can be used to garner additional information on the biodegradability of chemicals to assess persistence and avoid the need for higher tier simulation testing.

3.14.P-We124 Modifications of Simulation Study Test Design to Improve Testing of Chemicals and Biocides

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The calculation reliable degradation half-lives is of utmost importance for the persistency assessment of a chemical. To determine the transformation and corresponding degradation half-lives of chemicals in environmental compartments like soil, sediment and water, simulation studies acc. to OECD test guidelines 307, 308 and 309 are requested by the authorities. These guidelines were developed about 20 years ago, with the focus on pesticide regulation. Hence the general procedures of these studies mainly consider the properties of pesticides. To derive reliable degradation half-lives, the experimental approach of a simulation study must consider the physico-chemical properties and degradation behaviour of the substance to be tested. As the physico-chemical properties and degradation behaviour of chemicals and biocides often differ significantly from pesticides, the “standard” test design, used longtime for pesticides, is often not suitable for these substances. Especially for volatile substances and substances with fast mineralization, adaptations and improvements of the test design are necessary. Modifications of the test design to increase the reliability of degradation half-lives will be presented.

3.14.P-We125 Data Criteria Used for PBT/VPvB Assessment in the Community Rolling Action Plan (CoRAP) Substance Evaluations Under REACH

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Substances that do not readily breakdown in the environment and have the potential to bioaccumulate in organisms and biomagnify through food webs may pose a potential risk to human health and the environment. Managing risks from such substances to ensure safe, sustainable use is challenging. Under the REACH regulation, substances that are persistent, bioaccumulative and toxic (PBT), and very persistent, very bioaccumulative (vPvB) substances are identified as Substances of Very High Concern (SVHC) and are prioritised for risk management. Specific screening and assessment criteria for persistence, bioaccumulation, and toxicity are detailed in REACH Annex XIII, including the need for a weight-of-evidence determination. This requires all available relevant information to be considered in a single determination for the identification of a PBT or a vPvB substance. The variety and complexity of data which may be available for a substance and considered within a weight-of-evidence determination can inevitably pose a challenge for data evaluation and PBT identification. Extensive guidance is available under REACH to support assessment of substances. This is updated periodically as regulatory and scientific developments occur. In addition, there have been efforts made recently to improve the guidance available for weight-of-evidence determinations, to facilitate consistency, transparency, and predictability in decisions (OECD, 2019; ECHA, 2020; Suter et al., 2020). We describe here a retrospective, systematic appraisal of data used for PBT identification under REACH, to provide further insight on the types of data used in the decision-making process, how a weight-of-evidence was applied and to inform development of further guidance. Publicly available data for previous substance evaluations have been examined against the detailed guidance set out by ECHA and the wider scientific literature, to identify critical data quality criteria with regards to adequacy, reliability and relevance used in decision making. The approach to weight of evidence determination for each substance has been captured. We describe an overview of our analysis, and where relevant, opportunities for improving PBT/vPvB assessments. Such information can also be used by scientists generating test data to improve the likelihood that their information can be used in a regulatory context.

3.14.P-We126 Transformation Products of Pharmaceuticals - Comparison of Predictions With Observations in Environmental Simulation Studies

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Active pharmaceutical ingredients (APIs) and their transformation products (TPs) are commonly found in the environment. However, the environmental fate and risks are known for a relatively small portion of these. The use of prediction software, such as enviPath [1], could facilitate the identification and risk assessment of TPs. These tools are able to recognize functional groups and predict reactions based on existing biotransformation rules. However, sensitivity and selectivity of predictions are expected to be low because of missing rules and prediction of irrelevant TPs due to combinatorial explosion, particularly when applied to chemical moieties not previously contained in the underlying databases. In this work, we aimed to compare predicted TPs from different pathway prediction algorithms to experimentally observed TPs in biotransformation tests run with activated sludge from a municipal wastewater treatment plant. For this purpose, we selected 46 APIs that were structurally diverse, and for which data from standard soil and water-sediment studies (OECD 307/308) were available. By analyzing samples from batch experiments with LC-HRMS/MS, we will identify TPs using suspect and non-target screening. Experimentally obtained degradation pathways will be compared to predicted ones to identify missing transformation rules, indicated by non-predicted TPs, and discrepancies in probabilities for existing transformation rules. Further, the comparison of TPs between sludge experiments and standard OECD 307/308 will allow us to assess if biotransformation pathways observed in sludge biotransformation experiments reflect those observed in soil and water-sediment simulation tests. We expect to observe several unreported TPs, which were readily predicted by *in silico* methods, and anticipate to improve current prediction methods. Since the tools are freely accessible, they will support scientists, regulators and manufacturers to estimate environmental fate with little effort. [1] Wicker et al. Nucleic Acids Res. 2016, 44 (D1), D502–D508. Acknowledgment: PREMIER has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 875508. This Joint Undertaking receives support from the European Union’s Horizon

2020 research and innovation programme and EFPIA (<https://www.imi.europa.eu/>). Disclaimer: This abstract only reflects the authors' view and the JU is not responsible for any use that may be made of the information it contains.

3.14.P-We127 Improving Available Guidance for Persistence Assessment of Substances (CEFIC-LRI ECO52) - Using Overall Persistency in a Weight-Of Evidence for Persistence Assessment

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Chemical persistence plays a key role in the determination of environmental exposure making it an important component in risk assessment and regulation. However, existing frameworks under which persistence is assessed have shown some limitations. Chemical degradation rates are prone to wide variability depending on environmental conditions, which is difficult to address in a weight of evidence approach. In addition, many substances are problematic, or fall outside the applicability domain of existing frameworks due to their specific characteristics. Finally, evaluating degradation half-lives using a compartment-by-compartment approach is overly simplistic, and neglects dynamic multimedia exchanges and degradation processes that may have an important bearing on the overall persistence of a substance in the environment. This CEFIC-LRI project aims to address these issues by improving available guidance for persistence assessment of substances, including those considered problematic to evaluate under the current frameworks. Multi-media fate modelling (e.g. Mackay Level III) can in principle support weight-of-evidence (WoE) for persistence assessment especially with focus on justification for compartment of specific concern as well as on identification of remaining concern in untested compartments. However, according to the current guidance R.11 the distribution modelling results should only be regarded as qualitative or semi-quantitative and a case-by-case evaluation is needed. In this project, the overall persistency approach as well as dynamic multimedia fugacity box models (Level IV) has been evaluated to get a better understanding on persistence. The aim is to evaluate how multimedia modelling and overall fate can be incorporated to improve the robustness of current persistence assessment methodologies. Sensitivity analysis as well as case studies will be presented which illustrate the options how to use multimedia modelling in a weight-of-evidence approach.

3.14.P-We128 Regulatory and Technical Challenges of the Interpretation of Non-Extractable Residues in Persistence Assessment

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Biodegradation simulation tests provide key information in the assessment of the Persistence of a substance. Information is generated on the degradation of the parent substance and formation of metabolites and non-extractable residues (NER). Based on the non-ability to extract a fraction of residues with use of aggressive extraction procedures, NER have been regarded as a non-bioavailable sink in the environmental matrix. This interpretation prevailed when Persistence criteria were developed for regulatory purposes. The ECHA guidance on PBT assessment reflects a concern for NER to remobilize. The inclusion of NER in kinetics modeling has implication on the derivation of DT50 via introduction of a discrepancy between the current assessment approach and that used when the criteria were originally established. Consideration of NER-Type 1 as parent substance in kinetics modelling artificially increases the DT50 when compared to the P/vP cut-off criteria set in 2006. In addition, many technical challenges still remain to be addressed before any final decision can be made on the best way forward. While some extraction methods such as pressurized liquid extraction (PLE), Silylation and/or EDTA extraction can be suitable, none of them have been validated in this context. From experience, it is impractical to rely only on a non-standardized guidance document. There is a need for method standardization and inter-laboratory validation to increase method reliability (including among laboratories) prior to inclusion of agreed approach(es) in regulatory guidelines. Finally, the method to be applied will be very dependent on the substance and/or the chosen test method. Eventually, a non-mandatory, stepwise approach could be reasonable if it allows for flexibility (e.g. choices according to the needs and evidence already available). Conclusively, round-robin tests would be helpful to gather more insight into whether the various proposed extractions are suitable and feasible in the context of NER investigation and evaluation. Once the details are resolved and specific investigations are performed on a broader basis at the technical level, the results can be placed into a context potentially suitable for the assessment of persistence. Inclusion of historical substances used in the derivation of the P screening criteria in the validation of the testing approach would provide insights into the implications for assessing persistence.

3.14.P-We129 Integrating Bioavailability Measurements in Persistence Assessments of Partially-Biodegradable Organic Chemicals in Soil

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The integration of bioavailability assessments in standardized procedures for monitoring the biological transformation of organic chemicals in soil would help to explain biodegradation rates, leading to more realistic assessments of persistence and risk, especially with partial transformation reactions. We prospected this integration through a two-steps procedure: the first one was to follow the biodegradation (by the OECD 307 guideline) of ¹⁴C-labelled chemicals (pyrene –a PAH- and carbamazepine-a pharmaceutical compound) under solid-phase conditions leading to partial transformation products. The second step was to assess bioavailability of the parent chemicals and the metabolites at different stages of this incubation, using a standardized method (ISO 16751:2020) with soils samples at the start and the end of the incubation. This ISO method relies on the amount of contaminant

present in the matrix that can be released to the aqueous phase after 20 h in a well-mixed water soil mixture and in the presence of a receiving hydrophobic phase (Tenax). Because the ISO method was originally designed for non-polar compounds with $K_{ow} > 3$, the method was slightly adapted to carbamazepine ($K_{ow} = 2.7$), by changing the soil/water/Tenax ratio and the extraction period. The combined use of liquid scintillation and HPLC fractionation allowed the determination of the phase distribution in our system of the ^{14}C -labelled parent compound and metabolites among soil, water and Tenax. In the case of pyrene, the cometabolic transformation carried out by the microbial inoculum (*Pseudomonas putida* G7 cells, Fernández-López, et al., 2021) in the soil accounted for 40 % of the initial concentration (4 mg/kg), from which only 3 % were present as hydrophilic transformation products that were not trapped by Tenax but partitioned into the water. The rest remained as non-bioavailable residues. However, the bioavailable pyrene fraction in soil passed well to the Tenax and decreased as long as cometabolism proceeded, closing well the mass balances (>90 %). These results indicate that cometabolism decreased efficiently the risks from pyrene in the soil. The same approach was employed with carbamazepine, obtaining very interesting preliminary results on the phase distribution of this pharmaceutical compound and its metabolites in soil.

3.14.P-We130 Harmonization of EU Chemicals Hazard Assessment Via the Envisaged CLP Revision - What Is the Impact on Plant Protection Products (PPPs)?

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As part of the EU Commission “Chemical Strategy for Sustainability Towards a Toxic-Free Environment” a revision of the Classification, Packaging and Labelling (CLP) regulation is planned, including the introduction of new hazard classes on Persistent, Bioaccumulative, Toxic (PBT) and very Persistent, very Bioaccumulative (vPvB) substances, as well as Persistent, Mobile, Toxic (PMT) and very Persistent, very Mobile (vPvM) substances. This results in the implementation of an overarching hazard assessment tool, applicable across all chemical legislations, e.g. for industrial chemicals, biocidal products and plant protection products (PPPs), but ignores legislation-specific differences for derivation of trigger endpoints and consequences of a potential hazard classification. For PPPs, assessment criteria according to DG SANCO PBT/vPvB guidance (SANCO, 2012) are currently applicable, which, however, would be displaced by REACH Annex XIII PBT/vPvB guidance criteria with the planned CLP revision. On the one hand, this would lead to the applicability of REACH P/vP criteria (e.g., half-life (DT_{50}) values normalized to 12°C, consideration of metabolites and non-extractable residues (NER)), deviating from SANCO P/vP criteria (e.g., reference temperature for DT_{50} derivation of 20°C, no consideration of metabolites and NER). On the other hand, an additional hazard class for mobility will be applicable for PPPs in the frame of a PMT/vPvM assessment, ignoring the fact that a thorough groundwater risk assessment for PPPs is already in place. With mobility criteria currently under discussion, solely relying on adsorption properties ($\log K_{oc}$), this would lead to a high proportion of PPPs classified as “M” or “vM”. Consequently, the application of REACH Annex XIII criteria would lead to a high proportion of non-approved PPPs classifying as “PMT” or “vPvM” and to a significant number of PPPs classifying as “Candidates for Substitution” (CfS). We will provide data to highlight the impact of the planned regulatory changes on the authorization of PPPs. Overall, these developments result in a high degree of uncertainty, especially for the applicants of PPPs (but also for European and Member State authorities, farmers and citizens). There is no clarity for the applicant on how a P and M assessment for PPPs should be performed in the future with hazard classes and criteria implemented according to the current proposal.

3.14.P-We131 Harmonization of EU Chemicals Hazard Assessment Via the Envisaged CLP Revision - What Is the Impact on Plant Protection Products (PPPs)? 2

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As part of the EU Commission “Chemical Strategy for Sustainability Towards a Toxic-Free Environment” a revision of the Classification, Packaging and Labelling (CLP) regulation is planned, including the introduction of new hazard classes on Persistent, Bioaccumulative, Toxic (PBT) and very Persistent, very Bioaccumulative (vPvB) substances, as well as Persistent, Mobile, Toxic (PMT) and very Persistent, very Mobile (vPvM) substances. This results in the implementation of an overarching hazard assessment tool, applicable across all chemical legislations, e.g. for industrial chemicals, biocidal products and plant protection products (PPPs), but ignores legislation-specific differences for derivation of trigger endpoints and consequences of a potential hazard classification. For PPPs, assessment criteria according to DG SANCO PBT/vPvB guidance (SANCO, 2012) are currently applicable, which, however, would be displaced by REACH Annex XIII PBT/vPvB guidance criteria with the planned CLP revision. On the one hand, this would lead to the applicability of REACH P/vP criteria (e.g., half-life (DT_{50}) values normalized to 12°C, consideration of metabolites and non-extractable residues (NER)), deviating from SANCO P/vP criteria (e.g., reference temperature for DT_{50} derivation of 20°C, no consideration of metabolites and NER). On the other hand, an additional hazard class for mobility will be applicable for PPPs in the frame of a PMT/vPvM assessment, ignoring the fact that a thorough groundwater risk assessment for PPPs is already in place. With mobility criteria currently under discussion, solely relying on adsorption properties ($\log K_{oc}$), this would lead to a high proportion of PPPs classified as “M” or “vM”. Consequently, the application of REACH Annex XIII criteria would lead to a high proportion of non-approved PPPs classifying as “PMT” or “vPvM” and to a significant number of PPPs classifying as “Candidates for Substitution” (CfS). We will provide data to highlight the impact of the planned regulatory changes on the authorization of PPPs. Overall, these developments result in a high degree of uncertainty, especially for the applicants of PPPs (but also for European and Member State authorities, farmers and citizens). There is no clarity for the applicant on how a P and M assessment for PPPs should be performed in the future with hazard classes and criteria implemented according to the current proposal.

3.14.P-We132 Assessment of the Persistence of Ionic and Ionisable Organic Chemicals Under REACH

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For the protection of humans and the environment, the identification and regulation of chemicals with persistent (P), bioaccumulative (B) and toxic (T) properties are a central element within the environmental assessment. The criteria for the identification of PBT substances under REACH (Registration, Evaluation and Authorization of Chemicals) and the guidance for the PBT-assessment have been developed for neutral organic molecules. Thus, it is unknown, if the current assessment strategy for PBT substances is applicable on ionic and ionisable compounds. Due to their charge, ionic and ionisable substances behave differently in the environment compared to neutral substances. Cationic, anionic and amphoteric chemicals change their characteristics (e.g. water solubility, log K_{ow}) as a function of the environmental pH and their acid and base dissociation constants. Consequently, the distribution of these substances within the environmental compartments, e.g. portions of the chemicals being dissolved in the water phase and adsorbed to solid phases, are affected. This, at the same time, will influence the bioavailability of ionic substances for potentially decomposing microorganisms, governing their biotic degradation and persistence. In order to improve the evaluation of the persistence of ionic and ionisable substances, we compared the fate and the distribution of an anionic and cationic substance compared to a chemical without any charge with identical molecular core structures. The distribution of three different ¹⁴C-labelled chemicals within a soil test system according to their mineralized, extractable and non-extractable portion were determined. The influence of positive and negative charges on the biodegradability will be elucidated.

3.14.P-We133 New Approach for Consideration of Non-Extractable Residues (NER) in Persistence Assessment Suitable in Regulatory Practice

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Non-extractable residues (NER) are formed in transformation simulation tests in soil and sediment according to OECD 307, 308 and 309. Besides substance properties and soil/sediment type, the amount of NER strongly depends on the extraction methods. However, a standardised extraction procedure for determination of total NER is lacking. Thus, the boundary between extractable fraction and NER is currently not precisely specified. From a regulatory view, NER are of significance for persistence assessment to identify PBT, vPvB, or POP substances. NER are formed by various processes. They can be reversibly bound by adsorption or physical entrapment (**type I**) and pose a potential risk to the environment (hidden hazards). But they can also be covalently bound to organic matter (**type II**) or transformed into biomass (biogenic NER, **type III**) without risk to be remobilised again (safe sink). Up to now, a standardised or commonly accepted method to distinguish between the different NER types does not exist, but has been in the focus of ECHA (NER discussion paper 2018) and the German UBA (BfG project). These approaches differ to a certain extent. BfG proposes a pragmatic, standardisable procedure without further chemical analysis of the different extracts. The ECHA approach focuses on NER specification and analytical determination of bio-NER, sequestered, and covalently bound NER. In 2018, UBA funded an additional project to compare the existing approaches and testing strategies and to check for practicability. The aim was to develop a refined approach to assess their hazard potential with respect to the general applicability for an adequate persistence assessment including NER. For this purpose, simulation tests in soil (OECD 307) with the ¹⁴C- and ¹³C-labelled substances Bromoxynil, Isoproturon und Sulfadiazine were carried out. Different extraction methods were applied aiming to develop a standard procedure for total NER determination. Subsequently, EDTA extraction, silylation and acidic hydrolysis had been performed and compared in order to characterise the different NER types formed. In February 2021, UBA and its research partners organised an EU-wide workshop to present the results of the project and two proposals for the consideration of NER for discussion with scientists, stakeholders and regulators. Taking into account the comments from the workshop and a subsequent public consultation, one harmonised approach suitable for regulatory practice has now been developed.

3.14.P-We134 Insights Into Biodegradation of Functional Polymers Using Omics

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Functional polymers are used in many applications in our daily life as for example in detergents and cosmetic products. These materials are normally discarded down the drain and are treated in waste-water treatment plants. Therefore, a fundamental understanding of their biodegradability is of great importance. OECD testing guidelines are normally used to investigate the persistency of substances. However, these guidelines have been historically developed for small molecules and adaptation might be required for polymeric materials. In this work we investigate the biodegradation of a model class of functional polymers with OECD 301 F test and adaptations thereof. In addition enrichment experiments combined with metagenomics and metatranscriptomics are utilized to elucidate the biodegradation process of selected readily biodegradable functional polymers. Through this method we can identify which microbes are responsible for the biodegradation of the polymer, what oligomeric/monomeric units are taken up by the microbes and which biochemical pathway is responsible in the microorganisms to digest the polymer. Using the metagenomic results we further investigate the mechanism of degradation of the polymers with the help of metaproteomics and other analytical techniques. Using this approach, we can demonstrate that synthetic polymers such as the ones presented in this study are truly biodegradable. Furthermore, our results prove the potential to use omics to investigate

biodegradability of polymeric materials, to understand which microbes are involved in their biodegradation and what is the regulatory pathway through which the polymers are processed.

3.14.P-We135 ZeroPM - Substance Grouping

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In the H2020 Project ZeroPM – Zero Pollution of Persistent, Mobile substances, there will be work package devoted to Substance Grouping. Substance Grouping is seen as a key strategy to avoid “regrettable substitution” in which one hazardous substance in the market is substituted with another hazardous substitute. This is particularly the case where the new hazardous substance was structurally similar to the one that replaced it. There are several examples of this with decabromodiphenylether being replaced by decabromodiphenylethane, bisphenol a being replaced with bisphenol s, perfluorooctylsulfonate being replaced by perfluorobutylsulfonate, etc. This work package will identify all persistent, mobile and toxic/very persistent very mobile (PMT/vPvM) substances on the global market and develop a novel PMT/vPvM substance grouping approach to inform assessment, prioritization, substitution, regulation and removal. This substance grouping can take three forms, such as grouping based on common transformation products, grouping based on read-across of moieties that are causing the hazard and grouping based on extreme persistency leading. This work will start using case studies of suspected PMT/vPvM substance groups: small PFAS, triazines and triazoles, and later work towards expanding this work for other groups within the global chemical inventory. Examples include grouping all short-chain PFAS as a similar hazardous class, grouping precursors of melamine like cyromelamine with melamin, and non-persistent triazole containing compounds with persistent triazole transformation products. Acknowledgment: This project has received funding from the European Union's Horizon H2020 research and innovation program under grant agreement No 101036756.

3.14 Persistent, Mobile and Toxic (PMT/vPvM) substances: New Perspectives and Developments in their Assessment, Management and Regulation (Poster Corner)

3.14.PC-We19 Biotransformation of Chemicals in Water-Sediment Systems: Comparison of Low-Level Spiking and No Spiking in a Modified OECD 309 Test

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The OECD 309 test for studying aquatic biotransformation of chemicals in laboratory experiments has several shortcomings including poor reproducibility and questionable environmental relevance. To ameliorate these weaknesses, a modified biotransformation test system was evaluated using river water and sediment from 3 different rivers, Fyrisån (FYR), Enköpingsån (ENK), and Hågaån (HÅG), in Sweden. We modified 5 aspects of the test: 1. The experiments were started within 5 hours of sampling to minimize disturbance of the microbial communities; 2. The sediment concentration was increased to 50 g wet solids L⁻¹ to achieve more stable microbial communities in the reactor; 3. The incubation bottle was sealed to prevent changes in the ionization state of chemicals due to pH changes induced by outgassing of CO₂ from the oversaturated river water; 4. The amount of chemical spiked was reduced or the chemicals were not spiked at all to minimize the effect of spiking on the microbial community and biotransformation rate; 5. The length of the test was reduced to 14 days to minimize microbial community changes associated with long incubation periods. Three incubation groups with no spiking, 0.5 µg/L spiking, and 5 µg/L spiking were set up with five replicates in each group. We measured the dissipation of 56 substances in the FYR and ENK suspensions and of 80 substances in the HÅG suspensions. The dissipation kinetics of these substances are compared between the different incubation groups to see whether low-level spiking provides comparable results to no spiking as well as good reproducibility.

3.14.PC-We20 Effectiveness of Ready Biodegradability Tests As a Stringent Screening for Persistence in All Environmental Compartments

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The assessment of persistence under REACH is based on a tiered testing strategy (described in the PBT ECHA guidance R.11) which logically starts with cheap, fast and stringent methods, and gradually progresses to recommend more complex and time-consuming methodologies, if needed. The traditional starting point for testing biodegradability is the ready biodegradability test (RBT), like the OECD 301 series, which are considered so stringent that a “pass” level in a reliable RBT test is normally considered as sufficient information to conclude a substance as “not persistent” in any environmental compartment. Higher tier information on biodegradability would be generated by performing simulation tests (OECD 307, 308 and 309), which are compartment specific for soil, sediment and freshwater. This implies that a half-life leading to a conclusion of “not persistent” in a simulation test for a given compartment does not clear the suspicion of persistence in the other compartments, while a conclusion of “not persistent” in a RBT should be applicable to all environmental compartments. Here we determine the likelihood of a readily biodegradable substance being persistent in any environmental compartment by analysing a dataset of substances for which both RBT data and simulation test data exist, generated with the help of the OECD QSAR Toolbox and manual analysis of the data in ECHA’s database. The data collected underwent a quality check to flag tests which report half-lives but have issues casting doubts on their validity. Amongst 320 data points for simulation tests on substances with ready biodegradability

information reported, there were no substances that passed a ready biodegradability test for which reliable simulation data information would indicate persistence in any compartment. From those substances identified as “not readily biodegradable”, the vast majority were not persistent according to the half-lives reported, supporting the idea that the screening test is stringent enough to cover persistence in all environmental compartments.

3.14.PC-We21 Potential of Read-Across Approaches for the Biotransformation of Organic Contaminants Between Activated Sludge and Standard OECD 307 - 308 Tests

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The degradation half-lives (DT50) derived from the standard tests OECD 307 and 308 are commonly used to assess persistence of a chemical in soil and water-sediments systems. However, these tests are time- and resource-consuming compared to the experiments in activated sludge proposed in [1], which showed that DT50s of agrochemicals in soil could be estimated from 48h-tests in activated sludge. Our goal is to assess further the opportunities to read-across biotransformation kinetics observed in activated sludge to the soil and sediment-water compartments for a set of structurally diverse organic contaminants. We performed experiments using activated sludge from a local WWTP to study the biotransformation of 20 previously investigated agrochemicals and 48 newly added pharmaceuticals with available DT50s from OECD 307 and/or 308. Concentrations of the chemicals were measured using LC-HRMS to determine DT50s and screen for transformation products (TPs). Our outcomes with the 20 agrochemicals showed reproducibility of previous results[1] and consistency in sludge DT50 outcomes. We obtained the best predictions of the respective OECD 307-DT50s with models using the sludge DT50 and the experimentally determined Koc to correct for differences in bioavailability of the chemicals in soil vs sludge. Additionally, suspect screening of TPs suggests that the biotransformation pathways of the agrochemicals in activated sludge largely overlap with the observed biotransformation pathways in soil. Based on preliminary results showing some qualitative agreement between pharmaceuticals persistent in activated sludge and in OECD 308, we will investigate to what extent the read-across approaches trained for the agrochemicals are also applicable to estimate half-lives of pharmaceuticals in standard OECD 307/308 systems. We hope to demonstrate that under certain constraints, activated sludge biotransformation tests provide an opportunity for easier assessment of persistence of organic contaminants. [1] K. Fenner, et.al, 2020, ES&T, 54, 3148-3158 Acknowledgment: PREMIER has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 875508. This Joint Undertaking receives support from the European Union's H2020 research and innovation programme and EFPIA (<https://www.imi.europa.eu/>). Disclaimer: This abstract only reflects the author's view and the JU is not responsible for any use that may be made of the information it contains.

3.15 Plastics in the terrestrial environment: improving understanding of occurrence and impacts as analytics improve

3.15.T-01 Vertical Microplastic Distribution in Agricultural Soils After Long-Term Treatment With Sewage Sludge

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Microplastic pollution of terrestrial ecosystems and its potential impact on ecosystem services is increasingly perceived as a threat. While the number of studies demonstrating microplastic presence in agricultural soil under different management systems is increasing, there is still a considerable knowledge gap on the potential mobility of microplastics. Laboratory-based process studies indicate transport of microplastics. However, field investigations of microplastic distribution in the soil profile are still lacking which makes it challenging to understand their long-term fate and local impact. The objective of this study was thus to investigate the abundance and spatial distribution of microplastics in soil profiles from a long-term field experiment, including field plots exposed to 25 years of bi-annual sewage sludge application in contrast to field plots without fertilization. To this end, soil cores of 90 cm depth were taken and segmented into 10 cm thick layers, except the plough layer (0-20 cm). Polyethylene (PE), polystyrene (PS) and polypropylene (PP) microplastics below 2 mm size were then analysed using pyrolysis gas chromatography mass spectrometry after density separation and subsequent organic solvent extraction with 1,2,4-TCB and *p*-xylene. Microplastic abundance and differences between treatments were polymer-specific with PE being more abundant when sewage sludge was applied to the soil. In contrast, PS and PP were less strongly related to sewage sludge applications and detected in occasional single instances in both treatments. The detection of PE, PS and PP in control plots indicates that the area may be exposed to other more diffuse input sources, albeit at low levels. Notably, all microplastics were at least partly detected below the plough layer (0-20 cm). PE was detected in significant levels down to approximately 40-50 cm in the soil profile, which is likely attributed to transport processes within the soil profile. On the one hand, this emphasizes the need to consider deeper soil layers for accurate mass estimates and monitoring purposes; on the other hand, our results indicate that depending on the soil properties, soils may act as a temporary sink for microplastics. Further investigations on soil physical and biological properties are needed to understand the observed transport depth of microplastics and make reliable estimates on the temporal dimension of microplastic migration in the soil profile.

3.15.T-02 Plastic Aging in Soil

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The micro- (MP) and nanoplastic (NP) contamination of soil is of great importance as huge amounts of plastic are emitted in the environment and the yearly amount of MP added to agricultural soil alone exceed the amount emitted to the world ocean. Over

time, MP/NP in soils can be affected by physical, chemical and biological factors, which change their chemical structure, size, shape and surface properties. Most of the current methods of MP/NP identification rely on specific optical properties and chemical spectra. When the properties of plastic are altered by the aging of the material, these methods can fail to identify them properly, leading to an underestimation of the potential environmental issue of MP/NP. In order to understand how plastic age in soil, MP were collected from agricultural soils in Switzerland and homemade MP/NP particles of PE, PET, PA, PU and PC were incubated in two different soils for 3 months or 1 year. MP were microtomed into thin sections and the change of chemical composition from the surface towards the bulk material of the particle was analysed by STXM-NEXAFS. Additionally, their surface was investigated by acquiring SEM picture in order to characterize possible physical changes. **1. 2.** Several of the MP particles found in the agricultural fields showed a chemical variation along the gradient from the surface of the particles towards the bulk material. The transition between the surface structure to the bulk material occurs to some hundred nm. From the incubation experiment only the particles incubated for 3 months could be analyzed so far. For these particles no significant effect of the incubation could be observed, indicating that 3 month is too short to cause significant aging of MP surfaces. However, we expect changes of the surface of the particles to be visible after 1 year incubation and to change according to the soil used and the polymer incubated. The preliminary results show that the surface of MP changes its chemical structure while being buried in the soil. However, this process takes time and no changes of the surface could be observed after three month of incubation in two different soils.

3.15.T-03 When Do Micro-Or Nanoplastics Play a Role in the Relocation of Contaminants in Agricultural Soil?

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Insufficient waste management in combination with slow degradation rates contribute to the ubiquitous accumulation of micro- and nanoplastics (MNP) in environmental systems and particularly agricultural soils. Atmospheric transport and wind drift carry airborne MNP into soils but especially fertilization with compost or sewage sludge, containing high amounts of MNP, as well as degrading mulch films contribute to the plastic load in agricultural soils. MNP carry pollutants that have either been added as functional additives during the production, or have been passively sorbed during their life cycle. Therefore, concerns are frequently voiced that MNP transport the associated contaminants into deeper soil layers or even into groundwater. In this study we aim to elucidate whether and to what extent MNP facilitate the vertical transport of organic contaminants in agricultural soil. For this, we calculated the ratio between transport time of the MNP in soil and the desorption time of the sorbed contaminants by means of two diffusion models for MNP between 0.1-1000 μm . By reviewing diffusion coefficients and distribution coefficients for the most prevalent polymers and contaminants as reported in the literature, this study could show that at a flow velocity of 1 m/a in the soil the contaminants establish equilibrium with the surrounding soil phases too fast to be transported by MNP, regardless of the governing diffusion mechanism. Only under preferential flow conditions with a flow velocity of 1 m/h, MNP >100 μm may transport hydrophobic contaminants with $\log K_{ow} > 5$ beyond one meter soil depth. However, this requires that continuous preferential flow conditions prevail and that the particles are fully mobile therein. Although MNP constitute a pathway of organic contaminants to agricultural soils, this study suggests that particularly compared to the multitude of natural mobile colloids in soil, the role of MNP as contaminant vector is rather overestimated. Nonetheless, the introduction of MNP into agricultural fields requires stricter regulation as MNP that carry contaminants release these in the upper soil layers, inducing detrimental effects on soil organisms, crops and ultimately the consumer.

3.15.T-04 Long Term Contamination of Plastic Mulch and Pesticides Residues Effects on the Lettuce Growth

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In arid and semi-arid regions, the use of plastic mulch and pesticides in conventional agriculture is nearly ubiquitous. The most common plastic used for mulching is Low Density Polyethylene (LDPE). LDPE mulch needs to be removed after harvest and LDPE debris accumulates in the environment. Some plastic producers have tried to improve the degradation processes of plastic to avoid plastic mulch removal and plastic debris accumulation by adding pro-oxidant additives to LDPE, so called Pro-oxidant Additive Containing (PAC) plastics or by using biodegradable plastic polymers. However PAC plastics leave residues when ploughed into the soil and some biodegradable plastic don't degrade as fast as expected in the field conditions. Similarly pesticides are known also to accumulate in the soil. Studies investigating the effect of these contaminants on the soil fertility most often consider pristine plastic or single pesticides. We investigated the effect of one year incubation of plastic debris and pesticides in soil on the growth of lettuces in south east Spain. More precisely, we tested three plastic mulch residues and 3 commercial pesticides, including a treatment with the cocktail of the 3 pesticides compared to controls for a total of 20 treatments. The plastic mulches were first exposed 3 months to the summer weather in the field, cleaned, cut, grounded into macro- and micro-plastics debris and added in 1kg soil mesocosm at a 0.2% content w/w. Pesticides were applied 3 times, every 6 months, following the prescribed application doses. The 1kg soil mesocosms were left 1 year, protected from the wind and were irrigated to recreate farming conditions. After one year lettuces seedlings, *Lactuca sativa*, were planted in the incubated soil. The plant growth was assessed during the growing period (number of leaves, chlorophyll content, stomatal conduction) and at the harvest (fresh and dry biomass, leaf area, stem diameter). Preliminary results indicate that lettuces growing in soil incubated with the tested biodegradable plastic produced a significantly lower biomass compared to the control or the soil incubated with LDPE plastic.

3.15.T-05 Earthworms Ingest Microplastic Fibres and NanoPlastics With Effects on Egestion Rate and Long-Term Retention

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Microplastic fibres (MPFs) and nanoplastics (NPs) have the potential to be hazardous to soil organisms. Understanding uptake into organisms is key in assessing these effects, but this is often limited by the analytical challenges to quantify smaller-sized plastics in complex matrices. This study used MPFs and NPs containing inorganic tracers (In, Pd) to quantify uptake in the earthworm *Lumbricus terrestris*. Following seven days exposure, tracer concentrations were measured in earthworms and faeces. Earthworms exposed to 500 µg MPFs/g soil retained an estimated 32 MPFs in their tissues, while at 5000 µg MPFs/g earthworms retained between 2 and 593 MPFs. High variation in body burdens of MPFs was linked to soil retention in earthworms and reduced faeces production, suggesting egestion was being affected by MPFs. NPs uptake and elimination was also assessed over a more extended time-period of 42 days. After 1 day, NPs were no longer detectable in faeces during the elimination phase. However, some retention of NPs in the earthworm was estimated, not linked to retained soil, indicating not all NPs were eliminated. MPFs and NPs uptake can be quantified in earthworms and both particle types can be retained beyond the depuration period, suggesting the potential for longer-term accumulation

3.15 Plastics in the terrestrial environment: improving understanding of occurrence and impacts as analytics improve (Virtual Only)

3.15.V-01 Expression of Immune-Related Genes in Haemocytes of *Porcellio scaber* After Exposure to Microplastics in Soil: A CASE of Tyre Particles

Andraž Dolar¹, **Damjana Drobne²**, **Mojca Narat³** and **Anita Jemec⁴**, (1)Biology, Biotechnical faculty, Ljubljana, Slovenia, (2)University of Ljubljana, Slovenia, (3)University of Ljubljana, Biotechnical Faculty, Slovenia, (4)University of Ljubljana, Biotechnical Fac., Slovenia

Expression of immune-related genes in haemocytes of *Porcellio scaber* after exposure to microplastics in soil: a case of tyre particles **Andraž Dolar^{1*}**, **Damjana Drobne¹**, **Mojca Narat²**, **Anita Jemec Kokalj¹** ¹University of Ljubljana, Biotechnical Faculty, Department of Biology, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia ²University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia * presenting author Tyre particles, are produced by tyre wear and are one of the largest sources of microplastics to the environment, accounting for nearly 6 million tonnes of particles per year worldwide. Because these particles also contain a variety of potentially harmful chemicals (e.g., metals and organic compounds), this property could increase the risk of adverse effects of these particles on soil invertebrates. The aim of this study was to evaluate the expression of immune-related genes in the haemolymph cells (haemocytes) of woodlice *Porcellio scaber* after two different exposure durations (4-days and 14-days) to tyre particles of 1.5% w/w in standard natural soil Lufa 2.2. Tyre particles were produced from mixed end-of-life passenger car tires by cryo-milling and sieving to the size below 180 µm (mean size of 102.9 µm). Nine immune-related genes, namely catalase (*Cat*), manganese superoxide dismutase (*MnSod*), nitric oxide synthase (*Nos*), cyclophilin G (*CypG*), prophenoloxidase activating factor 2a (*Ppae2a*), Down syndrome cell adhesion molecule (*Dscam*), toll-like receptor 4 (*Toll4*), myeloid differentiation factor 88 (*MyD88*), and masquerade (*Mas*)-like protein, were selected to evaluate innate immune response of woodlice after exposure to microplastics. Relative gene expression of selected genes was calculated by the 2^{-ΔΔCt} method using β-actin and elongation factor 2 as reference genes. We observed changes in the expression levels of *Cat*, *MnSod*, *Nos*, *Toll4*, and *MyD88* genes in haemocytes of woodlice exposed to tyre particles in soil compared to the control group. After a 4-day exposure to 1.5% tyre particles in soil, the expression levels of the *Cat* and *Toll4* genes was increased, while after a 14-day exposure, we found increased expression levels of the *Nos*, *Toll4*, and *MyD88* genes and decreased expression of the *MnSod* gene compared with the control. These results showed that exposure to microplastics induce innate immune response of woodlice. Moreover, we found that a 4-day exposure to tyre particles significantly affected the number of haemocytes in the haemolymph of woodlice, especially granulocytes, while no change in haemocyte number was observed after a 14-day exposure. This indicates that organisms responded to exposure to tyre particles in soil, but did not cause adverse effects. This study has demonstrated the usefulness of gene expression analysis as a screening tool for assessing the response of *P. scaber* to various environmental stressors.

3.15.V-02 No Adverse Effects of Irregular Shaped Biodegradable Microplastics on the Reproduction of the Springtail *Folsomia candida* (Willem 1902) at Nowadays Environmentally Realistic Concentrations

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Microplastics (MP) are a novel set of entities with potentially harmful impact on wildlife worldwide. Effects are, for example, modulated by particle size, shape and polymer type. Especially for edaphic organisms the ecotoxicity of plastics is still poorly understood. Soil could be contaminated with plastics and also biodegradable plastics from various sources. For instance, fertilizers produced in bio-waste treatment plants often contain impurities like garbage bags made of a blend of polybutylene adipate terephthalate and polylactid acid (PBAT/PLA), resulting in the release of MP to soil. To investigate their influence on the standard soil arthropod *Folsomia candida*, we here conducted an OECD No. 232 reproduction test. For this, LUFA 2.2 standard soil was spiked with irregular shaped PBAT/PLA particles (D₅₀ = 73 µm with an IQR of 52 µm) using eight different MP concentrations ranging from 0.0043 to 1.5000 % [w/w]. It was hypothesized that the higher the dose, the greater the reduction in the number of offspring. Compared to the control, only the highest dose showed a biologically meaningful percentage change of reproduction of -34 % (95 % CI [-51 %, -18 %], Tamhane-Dunnett test: (t(10) = -3.15, p = .069). Note that the experiment was not valid as the

coefficient of variance of the number of juveniles in the control was 40 %. Overall, our results suggest that there is no dose-response pattern but only a relevant effect for very high MP concentrations that are much higher than nowadays common global concentrations of < 0.00045 %. Consequently, at recent exposure, short-term adverse effects of biodegradable MP on the reproduction of *F. candida* are very unlikely. To avoid ecological surprise in the soil ecosystem, future effect studies should also take into account the variety of MP, their manifold interactions with both biotic and abiotic factors as well as other endpoints like avoidance or stress markers and perform experiments with multiple species over many generations.

3.15 Plastics in the terrestrial environment: improving understanding of occurrence and impacts as analytics improve (Poster)

3.15.P-Tu167 An Alternative Method for Identification of Microplastics

Urška Šunta, Franja Prosenč, Mrs. Pia Leban, Polonca Trebše, Tjaša Griessler Bulc and Mojca Bavcon Kralj, University of Ljubljana, Faculty of Health Sciences, Slovenia

Microplastics (MPs) can be introduced in the terrestrial environment through atmospheric deposition, irrigation with wastewater, illegal waste dumpings, and the use of plastic in agriculture, such as mulching. For identification and quantification of MPs in soils, the soil samples should be pretreated using chemical and physical methods, such as drying, sieving, flotation, filtration, extraction, digestion, etc. After that MPs can be identified using destructive methods (pyrolysis gas chromatography-mass spectrometry (GC-MS), thermal desorption GC-MS) or non-destructive methods (ATR-FTIR and Raman spectroscopy, SEM). So far, none of the methods is standardised for the identification of MPs in soil samples, however, pyrolysis GC-MS, FTIR, and Raman spectroscopy are in the process of standardisation to use in the case of water and wastewater samples [3]. Not all laboratories have the necessary instruments to carry out qualitative analyses for MPs determination in environmental samples, therefore with this research, an alternative method is proposed for the determination of MPs in the sample that is based on headspace solid-phase microextraction and GC-MS analysis (HS-SPME-GC-MS). MPs of five polymer types (polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), polypropylene (PP), polyethylene (PE)) and in two size ranges (1-5 mm and 100 µm-1 mm) were used to develop the method. Based on the production of polymer characteristic compounds at the thermal degradation of MPs, and extraction of polymer specific fragment ions, it was possible to identify PET, PS, PVC, PP, and PE on their own, in polymer blend, in with MPs and microalgae spiked alluvial soil, and in a compost sample. The developed HS-SPME-GC-MS method shows the potential to become an acceptable alternative method for the determination of MPs in environmental samples.

3.15.P-Tu168 Green Surfactant-Free Synthesis and Characterisation of Palladium-Doped Polystyrene Particles for Environmental Fate Studies

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Recently, research efforts have increased to understand the fate and impact of plastics in the soil environment. Due to the background of natural organic matter in the environment, the quantification of polymers in environmental matrices is challenging. Therefore, the use of well-characterised model particles is mandatory to assess the fate of nanoplastics in soil. The quantification and localisation of these particles is alleviated by doping with metals with no natural background in the environment, e.g. palladium (Pd). In this work, we follow Mitrano et al.¹ and synthesise polystyrene particles in the range of up to 500 nm doped with Pd as tracer material. This synthesis has three stages: (I) synthesis of monodisperse spherical polystyrene particles according to Nichols,² (II) formation of Pd nanoparticles on the particle surface following Xu et al.³, and (III) coating of the doped particles with polystyrene to prevent Pd release and the change of the particle surface properties, which are key for the interaction in environmental matrices. We use only the essential educts styrene, divinylbenzene, K₂S₂O₈, Pd(NO₃)₂ and glucose to exclude effects from comonomers, contamination from reducing agents or detergents in environmental fate studies. The synthesised particles were characterised by light scattering methods and electron microscopy. The particles' Pd content and the potential Pd release were analysed by inductively coupled plasma mass spectrometry after digestion. The particles were successfully doped with 0.36% Pd nanoparticles and coated with polystyrene. Stability experiments show excellent colloidal stability with a zeta potential of approx. -70 mV and a stable hydrodynamic diameter of approx. 450 nm. Under light acidic conditions, the chemical stability of the particles was satisfactory with a Pd release 70.4%. This implied the good applicability of the synthesised particles for environmental fate studies. (1) Mitrano DM, Beltzung A, Frehland S, Schmiedgruber M, Cingolani A, Schmidt F. 2019. Synthesis of metal-doped nanoplastics and their utility to investigate fate and behaviour in complex environmental systems. *Nature Nanotechnol.* 14: 362-368. (2) Nichols CE. 1987. Preparation of polystyrene microspheres for laser velocimetry in wind tunnels. US NASA Technical Memorandum 89163. (3) Xu L, Wu XC, Zhu JJ. 2008. Green preparation and catalytic application of Pd nanoparticles. *Nanotechnology* 19: 305603.

3.15.P-Tu169 Microplastics in Composts - Identification and Quantification of Microplastic Contents Using a Thermal Decomposition Method

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The ubiquitous presence of unintended plastics in the environment has been an issue in scientific studies and public debate. Recent studies on MP findings are focused mainly on aquatic systems, while little is known about MP in terrestrial ecosystems. Fermentation residues, sewage sludge and compost are secondary raw material fertilizers and represent a possible input path of

plastics in soils. Soils are final sinks for micro plastics. In this context, samples were taken in a combined fermentation and composting plant in Germany to get real compost samples which were investigated. Existing regulations include requirements for total contents of plastics in combination with visual determination methods. In order to avoid possible underdeterminations, precise detection methods should be used in the future from a scientific point of view. For this reason, the use of thermoanalytical detection is an appropriate way. Spectroscopic methods such as Raman or FTIR are not suitable for determining the mass content of microplastic, as these output a particle number. In Germany, compost is a potential vector for MP in soil due to its use as fertilizer. Therefore, we show the application of ThermoExtractionDesorption-GasChromatography-MassSpectrometry (TED-GC-MS) as a fast, integral analytical technique, which in contrast to the spectroscopic methods does not measure the number of particles but a mass content. In a nitrogen atmosphere the sample is pyrolyzed to 600 °C and an excerpt of the pyrolysis gases is collected on a solid phase adsorber. Afterwards, the decomposition gases are desorbed and measured in a GC-MS system. Characteristic pyrolysis products of each polymer can be used to identify the polymer type and determine the mass contents in the present sample. For the first time the work represents a routine procedure for the determination of plastics in composts and fermentation residues. This current study will also give inside in various important aspects of sample preparation, which include a meaningful size fractionation, a necessary density separation regarding the removal of inorganic contents and at finally a homogenization.

3.15.P-Tu170 Towards Improving the Analytical Methods to Assess the Biodegradation of Microplastics in Soil Environments

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Microplastics are an emerging contaminant of concern to soil due to their small size and resistance to biodegradation. It is estimated that 36,000 tonnes of microplastics are intentionally released to the environment each year, which is equivalent to ~10 billion plastic bottles (ECHA, 2019). Many of these end up in agricultural soil which is a major sink for microplastic pollution; around 23,500 tonnes of microplastics are directly attributable to the agriculture and horticulture industries due to the application of controlled release fertilisers, fertiliser additives, treated seeds, and capsule suspension plant protection products (ECHA, 2019). This direct application of intentionally produced microplastics is preventable, thereby leading to restrictions on their use by the European Chemicals Agency, via the phased replacement and restriction of single-use plastics. Within the new restrictions, it is specified that biodegradable polymers can be used as alternatives to traditional plastics where appropriate. This has led to the development of biodegradable alternatives for use in pesticide applications. Biodegradable plastics are degradable by microorganisms and undergo significant changes in chemical structure under specific environmental conditions (aerobic conditions in the dark at 20 °C with degradation within 120 days). This gives the expectation that biodegradable polymers should be completely biodegraded by microorganisms into carbon dioxide, water, and organic material, with no fragmentation into smaller microplastics or nano-plastic particles under standardised test conditions, although methods to confirm this are not yet available. To ensure that newly developed polymers are fully biodegrading in the environment, standardised analytical methods are required that can accurately identify and quantify microplastics/polymer fragments in the soil environment to assess the level of biodegradation in conjunction with standard tests for biodegradation. This poster outlines how the adaptation of current soil analytical methods for use with biodegradable microplastics, and how these could be used in conjunction with standard biodegradation tests (ISO 17556 and OECD 307) to ascertain if newly developed polymers for use with crop protection products are completely biodegrading in the soil environment.

3.15.P-Tu171 What Is the Impact of Agricultural Mulch Films on Terrestrial Ecosystems?

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As plastics production continues to grow worldwide, plastics in the environment are becoming increasingly relevant. In the “iMulch” project, the effect of agricultural mulch film on soil ecosystems and organisms using different perspectives were analysed. A Method for the analysis of microplastics in soil samples was developed for Raman spectroscopy and Thermo extraction desorption Gas chromatography mass spectrometry (TED-GCMS). The aging of the mulch film was analysed in laboratory and mesocosm soil test and additionally in a laboratory sewage treatment plant. The transport and fate of the mulch polymers in soils using ¹⁴C marked polymers in outdoor lysimeter experiments was investigated- Experiments examining effects of the mulch fragments on organism were conducted. The “upcycling” of mulch films by bacteria was investigated. A life cycle assessment of conventional and biodegradable agricultural mulch films was conducted. An avoidance and substitution strategy was derived from the results with the aim of reducing plastic film fragments in the environment. This presentation will give you an overview of the results of this project.

3.15.P-Tu172 Fate and Effect of Doped and Non-Doped Microplastic Particles Under Environmental Relevant Conditions in an Outdoor Lysimeter Study

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Plastics in the environment are considered one of the key challenges of the 21st century. The application of sludge to agricultural soils as fertilizer will lead to microplastic (MP) contamination of soils. For a comprehensive risk assessment information on the fate and effect of MPs in soil is needed. There is a lack of information for the terrestrial environment, because detection and speciation of MP in soil is extremely challenging. Thus, the use of model particles is inevitable. Our aim was to investigate the fate and effect of polystyrene particles (PS-P) and polystyrene particles with a core of silver nanoparticles (Ag-PS-P) in a sewage treatment plant (STP) and a subsequent outdoor lysimeter study. Both particles were synthesised by emulsion polymerization and characterized by scanning electron microscopy (SEM) and dynamic light scattering (DLS). The particles were added into single STPs for 12 days following the OECD 303A. Samples were taken from sludge and effluent to determine the fate of the MPs. In addition, the effect on the microorganisms was investigated by measurements of the DOC elimination. After 12 days, the sludge was dewatered. To achieve three consecutive test concentrations, the sludge of the two treatments (PS-P, Ag-PS-P) was mixed with control sludge. Afterwards, 1.67 g dry matter sludge of each treatment were added per kg dry soil. Three replicates of 5 kg dry matter soil were prepared for each treatment. The replicates were incubated outdoor and wheat was sowed on each lysimeter. Over 4 month leachate samples were taken at regular intervals and the effect on soil microorganisms (substrate induced respiration (SIR), potential ammonium oxidation (PAO)) was examined. After dialysis and dilution, all particles were present as single particles, which was confirmed by DLS. The SEM pictures showed that one silver core particles was centrally located in most of the PS-P. No impact on the DOC elimination of the STP due to the MPs was observed. There were no PS-P detected in the effluent samples by SEM, indicating that most of the particles were retained by the sewage sludge. This could be verified by analysis of the pure sewage sludge and soil samples. Single particles were detected non-aggregated in the sewage sludge and soil. There was no effect due to the PS-P on SIR and PAO throughout the 3-month exposure period. The Ag-PS-P affected the PAO (inhibition < 20%) after long-term incubation indicating a toxic effect due to released Ag ions.

3.15.P-Tu173 Validation of Microplastic Accumulation in Agricultural Fields Receiving Wastewater Sludge

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Microplastic (MP) are formed when plastic items are exposed to wind, weather, and wear. Some accumulate on urban surfaces and are washed off during rain and conveyed to wastewater treatment plants (WWTP). MP are also generated in households and industries and conveyed to the WWTP. This leads to high levels in the WWTP sludge. When agricultural fields are treated with such sludge, a possible pathway for MP accumulation in soil is created – the extent of accumulation is however still largely unknown. This study investigates the occurrence of MP in the size range of 5000 to 10 μ m in agricultural fields that have received WWTP sludge, with the objective to increase the knowledge on, and determine the accumulation of, MP in such fields. Samples were collected from Copenhagen University's CRUCIAL test fields in Taastrup and two other Danish fields. Two CRUCIAL fields that had received WWTP sludge were sampled intensively by taking 12 samples in total. Here the spatial variation of MP over short and longer range is accounted for. To serve as a baseline for background MP concentration in soils, samples were collected from two CRUCIAL fields which had not received sludge and the two other agricultural fields. Approximately 280 g of soil was treated for each sample location to extract MP. Five basic steps were applied: Oxidation, flotation, enzymatic treatment, second oxidation, and second flotation. The extracts were analysed using FTIR imaging for particles < 500 μ m, where after the software siMPle was used to identify and quantify MP. Particles > 500 μ m were analysed by ATR-FTIR. Of the detected MPs more than 95% were of acrylic, acrylic paint, polyamide (PA), polyethylene (PE), polyester, polypropylene (PP), polystyrene (PS), polyurethane (PU), polyvinylchloride (PVC). The most abundant polymer type was PP. As an example of results, the MP concentration in one field that had received sludge varied by roughly one order of magnitude, from 7586 MP to 47689 MP pr. kg, at sampling points just 13 meters apart.

3.15.P-Tu174 Impact of Doped and Undoped Polystyrene Particles on Soil and PLANTS

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Impacts of microplastic (MP) on the terrestrial environment have been largely overlooked. This might be explained by the general lack of adequate analytical methods for extracting and detecting MP and nanoplastic (NP) in complex organic soil matrices. This methodical gap illustrates the urgent need of well-characterized model particles that can help to develop methods to investigate the fate and bioavailability of MP and NP in soil. According to Mitrano et al. (2019), quantifying and localising these particles in soil and plants will be improved by doping the particles with metals that have no natural background in soil, e.g. palladium (Pd). Here, we applied Pd-doped polystyrene particles (PS-Pd-PS-P) and pure polystyrene particles (PS-P) to soil to test their fate and impact in a long-term toxicity test for plants. The particles were synthesized in our lab by emulsion polymerization. Three different concentrations of the PS-P and the PS-Pd-PS-P were characterized. Subsequently, the particle dispersions were applied to a Cambisol and a Podzol. According to ISO 22030, the spiked soil samples were used in a chronic plant growth inhibition test with *Avena sativa* L. Moreover, the response of ammonium oxidizing bacteria (AOB) to the spiked soil samples was investigated (ISO 15685). The PS-P and the PS-Pd-PS-P were in the expected sub- μ size range and showed a high colloidal stability. This can be explained by their negative zeta potential. The particles occurred as non-homoaggregated particles in the soil matrix. The Pd-doping was detectable in the soil and in or at the plant roots which is why the particles are suitable to test their fate in soil and plants. The test on chronic toxicity in higher plants fulfilled all validity criteria. In each of the three test concentrations of the

Cambisol, no statistically significant effects were found on shoot length, shoot fresh and dry weight, shoot water content and fresh and dry weight of the flowers. No concentration dependent inhibition could be observed. The water content of the flowers in all approaches differed significantly from the control. For the Cambisol, the inhibition was between 19 and 49% for PS-Pd-PS-P while inhibition for PS-P ranged from 2 to 25%. For the Podzol, the inhibition was lower compared to Cambisol and ranged from 8 to 35% for PS-Pd-PS-P and from 2 to 21% for PS-P. The obtained inhibitions indicated a concentration dependent effect due to the particles. No effects on the AOB were observed.

3.15.P-Tu175 Identifying Factors Controlling Nano and Microplastic Uptake and Impacts on a Crop Plant: *Cucumis sativus*

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Terrestrial ecosystems might be the greatest recipient of plastic waste. It is estimated that the concentration of plastic fragments could be up to 6.7 % of the soil weight in highly polluted environments. Agrosystems are likely to be the most contaminated terrestrial system besides landfills, with multiple pathways for plastics to enter soils such as sewage sludge, wastewater and mulching and subsequently crop plants may be impacted. Currently, little is known about the fate and hazards of microplastics (MPs) and nanoplastics (NPs) in plants due to technical difficulties to analyze particles in complex matrices. In addition, more information is needed to determine how physicochemical properties and environmental factors affect the hazards of plastics. Consequently, this work aimed at determining the effect of different types of plastics varying in polymer (PS, PA, PE), particle size (nano vs. micro), shape (spheres, fragments, fibers) and weathering state (pristine vs. environmentally aged). The effects of varying exposure concentrations of MPs and NPs (0, 10, and 100 mg/kg) were assessed after 28 days in different plant growth scenarios (soil, sand, and hydroponics) on the development and metabolism of cucumber (*Cucumis sativus*). As anticipated, results showed that exposure scenario was critical to plastic toxicity. In particular, phytotoxicity was higher in hydroponic exposures (e.g., decreased foliar biomass and area up to 50 % and 80%, respectively) while effects were less prevalent when MPs and NPs were in both sand and soil. The influence of physico-chemical parameters of the particles after plant exposure in sand suggested that particle size was the main controlling factor, with MPs having no significant effects while NP exposure led to phytotoxicity symptoms. In particular, a 50% decrease in fresh root biomass was shown, suggesting that NPs could impact root growth. In ongoing work, nutrient and plastic concentrations in leaves will be assessed by ICP-MS and py-GC-MS respectively.

3.15.P-Tu176 Investigating the Link Between Above- and Below-Ground Plastic Pollution and Plasticiser Occurrence

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In addition to the physical impacts of plastic particles on terrestrial organisms, toxicity will likely arise from additives associated with the polymer matrix, such as plasticisers. Historically, phthalates have been the most widely used class of plasticiser. However, concerns and restrictions surrounding the use of certain phthalates in Europe have coincided with an increase in the use of emerging non-phthalate plasticisers over the past 15 years. Despite this, the occurrence and levels of these emerging plasticisers in the terrestrial environment remains unknown. Could it be that the successors to phthalates are accumulating in soils? The occurrence of plasticisers in soils has been linked with point sources (e.g. sites of intensive agriculture), but the impact of diffuse plastic litter associated with e.g. urban areas or mismanagement of plastic waste is less well-studied. Constraining the links between the profiles of plastic and plasticiser contamination in soils will grant insights into the sources of plasticisers in the terrestrial environment. However, to date there has been no published work regarding the co-occurrence of above- and below-ground polymer and soil plasticiser contamination. Our study utilises recent developments in the field of microplastics research (e.g. semi-automated analytical pipelines capable of characterisation and semi-quantification of microplastics), alongside established analytical techniques such as GC-MS, to investigate the nature of these links in field samples collected from a range of land uses. We have developed a method to simultaneously extract and quantify a suite of legacy (i.e. phthalate) and emerging plasticisers from soil samples. There is a lack of data regarding the terrestrial occurrence of plastics in the UK. There is no data on emerging plasticisers in UK soils, whilst surveys of phthalates across various land uses have been primarily limited to the phthalate DEHP. Thus, the samples collected during this study also provide a unique insight into the status of plastics, phthalates, and emerging plasticisers in the UK terrestrial environment.

3.15.P-Tu177 Plant Response to Microplastics From Conventional and Biodegradable Mulching Films

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Mulching film products have been developed for agriculture and horticulture to facilitate cultivation, reduce the need for plant protection products and improve agriculture profitability. Biodegradable films replacing conventional plastic cover films are typically not collected from the application site, but instead left to degrade in the soil. However, degradation rate in northern conditions is assumed to be slower than under ideal laboratory conditions, and consequently plastic particles may accumulate and act as sources of microplastics in the soil. Therefore, it is essential to assess the potential risks for the safe use of these materials and prevent degradation of soil quality and fertility in the long run. In this study, we examined the effects of mulching film particles (< 1 mm) on Chinese cabbage (*Brassica pekinensis*) seed germination and plant growth rate in controlled conditions. Assessment of plant response to three different microplastic types was performed using one conventional and two biodegradable

plastics both in concentrations of 0.1% and 1% with three replicate plants for each concentration. Plant biotests were performed on two separate occasions: immediately after microplastic addition in control soil and after two months of mixture incubation. Results from these experiments will be presented. This study is part of the project “Microplastics in agricultural soil – Sources, effects and reduction (MicrAgri)” funded by the Ministry of Agriculture and Forestry of Finland, from the Makera Fund.

3.15.P-Tu178 Circular Economy in Agriculture - the Case of Microplastics

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Continuing population growth is driving global food demand, with agricultural activity expanding to keep pace. The linear functionality (take-use-dispose) in agriculture is causing irreversible environmental problems, therefore, a transition to a circular economy is encouraged. E.g., treated municipal wastewater can be used for irrigation and by-products of wastewater treatment and bio-waste processing can be used as fertilisers and soil amendments. Microplastics have been found to affect soil microorganisms, nutrient cycling, and plant growth; however, the effects are varying depending on their size, concentration, shape, and composition. To evaluate the realistic effects, quantification and classification of plastic contaminants in these resources and the receiving agricultural soil are needed. In this study, small plastic particles (< 2 mm and >2 mm) in (1) organic fertilisers – biowaste compost and sludge; and (2) soil – irrigated with wastewater and soil fertilised with compost were quantified and classified. Organic fertilisers, such as sludge from the wastewater treatment plant and biowaste compost are highly contaminated with plastic particles, both larger (>2 mm) as well as microplastics (< 2 mm). Interestingly, compost samples contained more plastic contaminants as compared to sludge, namely between 607 and 1186 of plastic particles (>2 mm) per kg of dry weight (dw) compost vs. 235 per kg of dw sludge. Soil fertilised with compost exhibited elevated plastic contamination as compared to the control soil fertilised with mineral fertilisers. In tilled soil, the abundance was more than four times the number in the control soil (38 vs. 9 particles/kg dw soil), whereas, in soil that was not tilled, the difference was more than twofold (16 vs. 6 particles/kg dw soil). Interestingly, tilled soil, fertilised with compost contained more than twice as many plastic particles as compared to soil fertilised with compost that did not undergo tillage. Soil irrigated with wastewater, on the other hand, exhibited only slightly elevated concentrations of plastic contaminants as compared to the control soil (5.8 vs. 3.4 of >2 mm particles/kg dw soil, and 28.3 vs. 25.8 of < 2 mm particles/kg dw soil). The results of this study will contribute towards a better understanding of the plastic contamination in agricultural soil originating from resource reuse. Additionally, they will facilitate a better experimental design of plastic effect studies.

3.15.P-Tu179 Citizen Science to Investigate Plastic Degradation in the Soil Environment

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One of the 10 major environmental challenges facing our society is that of plastic waste with an annual production of around 360 million tons of which only 8% are recycled. Among the alternatives developed to face this issue is the synthesis of so-called biodegradable plastics. However, to date their biodegradation status is based on their complete degradation within three months in controlled conditions. In the wild, where they mostly end-up, many biotic and abiotic factors can influence the plastic degradation rate such as humidity, temperature or soil biogeochemistry. The issue of plastic pollution affects everyone as a citizen and consumer and therefore is an ideal subject to develop a citizen science approach. Furthermore, this approach is particularly effective in answering ecological questions at scales of time and space that cannot be covered by a research team under lab conditions making it possible to study the effect of geographic gradients over long periods of time. The PlastiZen project aim at involving participants in a scientific experimental design over a 3-month period. The objectives of the project are (i) to verify the biodegradability of this type of plastics (kinetics measurements) along a wide gradient of locations in France, (ii) to identify the controlling factors (soil texture, pH, organic matter, C/N, pluviometry, temperature, microbial activity) and (iii) to raise public awareness of the issues surrounding the consumption of single-use plastic and their end-of-life. With more than 100 participants to date, we have been able to highlight a wide diversity in the degradation rate of the “biodegradable” plastics ranging from a complete degradation (down to microplastic particles) in some locations to no visible degradation in some other places. After 3 months, the majority of the plastics buried by the participants were damaged but did not completely disappear. The main controlling factor identified in this study was the amount of cumulative rain over the considered period followed by microbial activity, temperature and to a lesser extent soil pH. Finally, another interesting aspect of such citizen science project is to be able to compile social science data about public perception and behavior related with plastic consumption and in particular highlight the evolution of participant habits after their participation to the program.

3.15.P-Tu180 Are Biodegradables Increasing or Solving the Microplastic Pollution? Correlating Particle Size, Copolymer Degradation, and CO₂ Generation During Biodegradation in Compost and Soil

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Bans, reporting and labelling requirements for intentionally added primary microplastics shall avoid their release into the environment during or after use. Biodegradable polymers are derogated from the restriction proposed by the European Chemicals Agency. Biodegradation is based on microbial oxidation and hydrolysis processes that result in the complete transformation of polymer carbon into biomass and CO₂. And yet, the ability to biodegrade in specific compartments is no excuse for littering. For intentional application in the environment, e.g. mulch films in agriculture, biodegradable polymers could contribute to the solution to the plastic pollution crisis – if they do not contribute to the issue: One may hypothesize that fragments of the original polymer part could form during the degradation processes, and/or that only certain components degrade. Part of the answer is provided by

isotope labeling of monomers, tracking their integration into biomass. Here we develop another sensitive tool by extracting and counting polymer particles during their biodegradation in compost and soil. Specifically, we studied biodegradation of PBAT-PLA copolymer blend and of PBSeT copolymer, and benchmarked them against LDPE as conventional non-degradable alternative. The materials were either dosed as 250 μm particles (considered as worst-case), or as 15 μm film (representing mulch film applications). Both industrial composting, relevant for packaging goods, and soil conditions, relevant for agricultural applications, were tested. During biodegradation (ISO 14855, ISO 17556), CO_2 evolution was monitored. Using a non-destructive and efficient extraction protocol, the particle number and size distribution, and the state of copolymer degradation were assessed via fluorescence microscopy and chemically selective Raman microscopy. Staining selectivity was additionally cross-checked by an independent incubation of a bespoke dye-labeled PBAT-PLA copolymer. Our results revealed that particle numbers dropped from the ISO-compliant initial dosing of about 80,000 particles per gram of compost to the limit of detection (LOD). At 90% CO_2 , more than 99.93% of particles have been removed. The time-resolved kinetics demonstrated that the degradation of first large, then small particles preceded the metabolism to CO_2 . Based on measured CO_2 release, and measured particle size fractions, we also inferred the kinetics of dissolved polymer as it is being metabolized.

3.16 Poly- and perfluoroalkyl substances (PFASs): Addressing Urgent Questions in the 21st Century (Part I)

3.16.T-01 Which of the (Mixed) Fluorinated, Chlorinated and Brominated N-Alkanes Are Persistent Organic Pollutants?

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Short-chain polychlorinated n-alkanes are ubiquitous industrial chemicals widely recognized as persistent organic pollutants. They represent only a small fraction of the 184,600 elemental compositions (C_{10-25}) and the myriad isomers of all possible (mixed) halogenated n-alkanes (PXAs). This study prioritizes the PXAs on the basis of their potential to persist, bioaccumulate and undergo long-range transport guided by quantitative structure-property relationships (QSPR), density functional theory (DFT), chemical fate models and partitioning space. The QSPR results narrow the list to 966 elemental compositions, of which 352 (23 Br, 83 Cl/F, 119 Br/Cl and 127 Br/F) are likely constituents of substances used as lubricants, plasticizers, and flame retardants. Complementary DFT calculations suggest that an additional 1367 elemental compositions characterized by a greater number of carbon and fluorine atoms, but fewer chlorine and bromine atoms, may also pose a risk. The results of this study underline the urgent need to identify and monitor these suspected pollutants.

3.16.T-02 Volatile PFAS in Air: Developing Methods for Targeted Analysis and Discovery of Non-Target Volatile PFAS

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Per- and polyfluorinated alkyl substances (PFAS) are analysed mainly by liquid chromatography, as traditionally the focus has been on monitoring PFAS in soil and water, targeting mainly the ionic PFAS species. Nevertheless, trace PFAS quantities in air is today also of concern from human health and environmental perspectives, and the analytical technology required by air monitoring scientists to address this area is already available and tested. Modern analytical TD-GC-MS systems were designed specifically for monitoring trace-level organic vapours and recent developments in automated TD technology have meant these methods can be applied to more and more challenging compounds. With the wide range of different chemistries as displayed within the wide-range of PFAS compounds, this TD technology can be implemented to monitor PFAS in air. The aim of this study was to evaluate the performance of the latest off-the-shelf sorbent tube sampling and automated TD-GC-MS analytical technology for analysing volatile and semi-volatile PFAS. This study focused on perfluoroalkyl carboxylic acids (C4 to C14), fluorotelomer alcohols (FTOHs), fluorotelomer acrylates (FTAcres) and fluorotelomer sulfonamides (FOSAs). These compounds have been shown to be widely distributed within indoor air, because of their high volatility and the wide use of PFAS compounds within everyday objects in our household. Additionally, in air the ionic species are less relevant. Often overlooked is the fact that also very volatile perfluorinated hydrocarbons (C1 to C3), also known as potent greenhouse gases and ozone depleting substances, are part of PFAS family. However, these require whole-air sampling using canisters, online monitoring or offline sampling bags and were not included in this study but have the potential to be included within the analytical system. Method development was carried out in the same manner as for all VOCs analysis. Guidelines from established methods, like the US EPA TO-17 can directly be transferred into this type of analysis. As single quadrupole MS with EI are often used for standard air monitoring methodology, this detector was chosen for the project, however it should be noted that other detectors may improve the sensitivity. Excellent method performance (linearity, repeatability in the order of 5% or less, storage stability tested up to 15 days, etc.) was demonstrated across the range of compounds tested, including low or sub-ppt detection limits for all compounds.

3.16.T-03 Total Oxidizable Precursors Assay for Human Serum: A Tool for Holistic Per- and Polyfluoroalkyl Substances (PFAS) Exposure Assessment

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Per- and polyfluoroalkyl substances (PFAS) are a class of industrial chemicals that have been detected worldwide in the environment, in wildlife and in humans. Even if over 4700 PFAS are registered in the market, only few of them are commonly analyzed in human samples and some fluorine mass-balance studies are showing that currently measured PFAS might not be

enough to describe the full extent of PFAS human exposure. Among the underlooked PFAS in human samples, precursors are of concern for human health because these compounds can be metabolized to perfluoroalkyl acids (PFAA) that are already known to be toxic. To look for both known and unknown precursors in human serum an adaptation of the total oxidizable precursors assay (TOPA) was developed. In the TOP assay precursors are converted to easy to measure PFAA by oxidation with heat-activated persulfate under alkaline conditions. The total oxidizable precursors are then quantified by measuring the PFAA produced by oxidation. The TOPA was introduced as a complementary tool for water analysis and had to be modified to be applied to small volumes of human serum samples. Modifications to the method were tested on reference serum samples spiked with model precursors. To fully convert the model substances the heating at 85°C had to be extended and a large excess of oxidant was needed. However, even when precursors were fully converted, the yield of identified oxidation products was not always 100%, ranging from 33 to 100%. As there can be precursors that are not at all or not fully converted to PFAA, care must be taken in quantifying the total amount of precursors present using the sum of PFAA produced by oxidation. Unequivocal information on the structure of the oxidizable part vanishes, but the distribution of products is a good indication of that, as well as of the structure of the original perfluorinated part. In general, the products are perfluorocarboxylic acids with the same or shorter perfluorinated chain, but for some precursors PFOS and perfluoroethercarboxylic acids can also be observed as oxidation products. The final method can be used to determine well known PFAS and oxidizable precursors using only one small serum aliquot. The TOP assay is a valuable tool to estimate the contribution of precursors present in human serum directly linked to human exposure, indicating their structure and type without requiring additional sample amounts, instrumentations or standards.

3.16.T-04 How Open and FAIR Cheminformatics Can Support the Discovery, Analysis and Assessment of PFAS

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The ever-increasing concerns about poly and perfluoroalkyl substances (PFAS) and calls for action upon them as a class has spurred intense debates on how to define and enumerate the “PFAS Chemical Space”. There are now a number of PFAS lists openly available to the community. These include the OECD PFAS list of ~4700 PFAS (ENV/JM/MONO(2018)7) and the US EPA PFASMASTER list of ~9000 PFAS. Over 30 more are available on resources such as the CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/chemical_lists/?search=PFAS) and the NORMAN Suspect List Exchange (<https://zenodo.org/communities/norman-sle/search?page=1&size=20&q=PFAS>). However, searching the large open chemical collection PubChem (<https://pubchem.ncbi.nlm.nih.gov/>; 111 million chemicals) reveals that ~6 million entries match the latest OECD PFAS definition where PFAS “contains at least one alkyl CF₂ group”. Navigating this space is challenging, between the tens of PFAS routinely monitored, thousands on existing lists, and the millions existing in databases. This work demonstrates how the cross-resource integration of information between the NORMAN Suspect List Exchange and PubChem can facilitate closing the gaps in major open databases and fast-track publication of new structures identified in research (*e.g.*, PFAS transformation products; previously undocumented PFAS) including detailed annotations such as reactions, spectral fragmentation patterns, collision cross section values and even NMR spectra. By highlighting and building on existing PubChem functionality, this in turn offers extensive bulk property calculations, patent information, available annotation (such as: uses, toxicity, and biomedical literature), download files, and advanced programmatic access. Since discovery of PFAS in the environment is critical, a relevant subset of PFAS in PubChem (~255K chemicals with –CF₂CF₂–) is made available as a PFAS database for non-target or suspect screening mass spectrometry efforts for incorporation into open software such as patRoan (<https://rickhilmus.github.io/patRoan/>), MetFrag (<https://msbi.ipb-halle.de/MetFrag/>) and more. A set of findable, accessible, interoperable, reusable (FAIR) templates are provided to enhance data sharing between resources and increase the availability of FAIR datasets for future data-hungry computational developments such as literature/patent mining, transformation product prediction (*e.g.* BioTransformer) and/or automated PFAS classification efforts.

3.16.T-05 Horizontal and Vertical Distribution of Perfluoroalkyl Acids (PFAAs) in the WATER Column of the Atlantic Ocean

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Perfluoroalkyl acids (PFAAs) are widely used synthetic chemicals and can be the degradation products of so-called precursors. They are characterized by unique physicochemical properties such as strong surface activity and stability. Another feature of PFAAs is that they are all mobile and highly persistent, which makes their occurrence in the environment problematic. Additionally, long-chain PFAAs are bioaccumulative and have been linked to adverse health effects in humans and wildlife. The oceans are thought to be the terminal sinks for these persistent substances but little is known about their fate in the water column. This study aimed to map the concentrations of PFAAs (with 6-12 carbon atoms (C₆-C₁₂)) in the surface and deep ocean, thus adding high quality analytical data to the currently limited database. Seawater depth profiles at 29 sampling stations were collected during the 29th Atlantic Meridional Transect (AMT29) cruise from the UK to southern Chile, and analyzed using high-performance liquid chromatography coupled with high resolution mass spectrometry. The generated data revealed a major PFAA input from the Mediterranean Sea through the Mediterranean Outflow Water that flows into the Atlantic Ocean. Elevated concentrations were observed in the English Channel as well. Another outstanding feature were accumulated concentrations of PFAAs at the eastern edge of the Northern Atlantic Subtropical Gyre. The highest PFAA median concentrations were measured in the Northern Hemisphere (n=119) with 61 pg L⁻¹, whereas for the Southern Hemisphere (n=77) a median concentration of 22 pg L⁻¹ was estimated. The composition at the surface was dominated by PFHxA, PFHpA, PFOA and PFNA. Trends with depth

within the water column were observable, showing that PFAA homologues with 6-9 carbons (C6-C9) dominated in surface waters, while longer-chain PFAAs (C10-C11) reached intermediate depths (500-1500 m). This is due to the increasing sorption affinity with increasing chain-length, so it is suspected that long-chain PFAAs sorb relatively strongly to organic matter and get transported downwards the water column.

3.16 Poly- and perfluoroalkyl substances (PFASs): Addressing Urgent Questions in the 21st Century (Part II)

3.16.T-06 The TOP Assay Uncovers Unknown PFAS in German Soils With Background Concentrations

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Per- and polyfluoroalkyl substances (PFAS) are highly persistent and have been found worldwide in many compartments. Their persistence in combination with toxic effects on environmental and human health have resulted in a ban of individual compounds and in an ongoing substitution by other PFAS. Today, PFAS are estimated to consist of more than 10,000 compounds including precursors and polymers making analysis of all single compounds impossible. To overcome this analytical gap, sum parameters, e.g. the total oxidizable precursor assay (TOP assay), have been developed and results indicate that the amount of unknown PFAS in the environment is in general higher than what is revealed by the analysis of individual well-known PFAS. Two studies were performed in Germany aiming to provide more information on the importance of precursors for PFAS background levels in soils as well as their spatial and temporal trends: Samples from the German environment species bank (time period 2002-2018) represent different ecosystem types of German soils. 20 top soil samples collected in North Rhine-Westphalia represent different land uses (agricultural land, grassland and forest). PFAS were detected in all soils and forestry samples showed highest concentrations before TOP assay. The soils from the specimen bank are often dominated by PFOS and PFOA. Sum concentrations of PFAS (C4-C13) range from 0.1 µg/kg for a agricultural used soil to 25 µg/kg in the organic layer of a forestry type location. The samples from the second study were also analyzed for ultrashort-chain PFAS such as TFA and PFPrA. The main components in these samples were TFA, PFBA, PFOA and PFOS. The PFAS concentrations (C2-C13) ranged from 0.8 -12.5 µg/kg and without TFA and PFPrA from 0.5 to 4.5 µg/kg. In the first study the increase after TOP assay varies strongly with sampling locations and years and ranges from no increase to an increase of 160 % for a low contaminated sample from an urban conurbation. In the second study most samples show much higher PFAS sum concentrations in soil after using TOP Assay. In some soils, also high increases of TFA concentrations were observed which may have another source than PFAS precursors, e.g. pesticides. The presence of unknown PFAS underlines the need for a more comprehensive strategy in PFAS regulation including precursors and substitutes and the prevention of further PFAS release to soils.

3.16.T-07 Perfluorinated Alkyl Substances Around Industrial Sites - Elevated Levels Can Cause Health Problems

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1. Introduction At several places in the world the production of per- and polyfluorinated alkyl substances (PFAS) has led to high levels of these compounds in the neighbourhoods of PFAS producing plants. In 2021, high levels of perfluorooctane sulphonic acid (PFOS) were found near a plant of 3M in Zwijndrecht, very close to Antwerp, Belgium. We analysed blood samples of inhabitants of Zwijndrecht, as well as local fish, sediment, surface water and drinking water. 2. Materials and methods Blood samples were taken from 16 persons that lived and/or worked within a few kilometers from the 3M site in Zwijndrecht. All persons completed a questionnaire in which they answered a series of questions about their background, age, gender, diet, etc. Flounder, marine vegetables, and surface water were sampled from the Western Scheldt at the Dutch/Belgian border and Antwerp harbour. Drinking (tap) water was sampled in Antwerp. A set of 17 PFAS was determined in all samples by LC-MS/MS analysis on an LC-Sciex Exion LC AD coupled to a Sciex 6500+ triple-quad MS, using an Xbridge BEH C18 XP 150 x 2.1 mm, 2.5 µm column and electrospray ionisation. 3. Results and discussion PFOS was dominant in all samples (2.1 – 1100 mg/L). The recent opinion on PFAS of the European Food Safety Agency (EFSA) gives a safe level of PFAS in human blood that is based on the sum of four PFAS congeners: PFOA, PFOS, PFHxS and PFNA. This sum should not be higher than 6.9 mg/L. The maximum value found was 1154 mg/L, which is 167-fold the norm. Most of the persons studied consumed vegetables or fruit from their own gardens and/or ate eggs from their own chickens. Based on these results (Table 1) a larger study on PFAS in human blood was initiated by the Flemish authorities in 800 persons living within a circle of 3 km around the 3M site. That study confirmed the here presented results. 90% of the PFAS concentrations found in the larger study exceeded the EFSA guidelines of 6.9 mg/L. Western Scheldt fish, sediment and water samples confirmed the PFOS pollution from Antwerp. The PFOS concentration in flounder was 24 mg/kg, lower than determined by Chu et al. [4] who found 322 mg/kg in 2006. The perfluoro-1-butane-sulfonamide (FBSA) detected by Chu et al. was also found by in our study. The results of this study urge much better inventories of current PFAS production plants.

3.16.T-08 An Outdoor Aging Study to Investigate the Release of Per- and Polyfluoroalkyl Substances (PFAS) From Functional Textiles

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Functional textiles were investigated for the emission of per- and polyfluoroalkyl substances (PFAS) via a real time outdoor weathering experiment in Sydney, Australia. Different side-chain fluorinated polymers (SFPs) (with C₄F₉SO₂-R, C₆F₁₃-C₂H₄-R

and C₈F₁₇-C₂H₄-R side-chains) were applied to polyamide (PA)-based textile fabrics and exposed on a rooftop to multiple natural stressors, including direct sunlight, precipitation, wind, and heat for up to 6 months. Thereafter, additional stress was applied to the fabrics via washing and abrasion. A multi-platform analytical approach was used for textile characterization which revealed losses of both low molecular weight PFAS and PFAS-containing textile fragments (e.g. microfibers) throughout weathering. The loss of color and water repellency of the textiles accompanied these changes. Targeted analysis showed that weathering led to formation of a distinct PFAA pattern for each SFP-based textile finish. Overall, this work provides evidence for two distinct emission pathways from SFP-coated textiles during weathering: a) loss and subsequent transformation of low molecular weight PFAA-precursors residuals, and b) loss of textile fibers/particles containing polymeric PFAS which could eventually lead to formation of persistent PFAAs in the environment.

3.16.T-09 PFAS in Drinking Water in Norway; Generally, Very Low Levels but Elevated Concentrations Were Detected Near Known PFAS Sources

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PFAS are contaminants that can be found in most ecosystems. Norway has had no production of PFAS, but PFOS have been used in fire-fighting foams and have been employed at a few industrial sites. In Norway, drinking water originate mostly from surface water. Norway has reported very few analytical measurements of PFAS in drinking water, and data was therefore warranted. Drinking water from 20 different drinking sources and 11 different waterworks were sampled and sent to NIVA for analyses of 31 PFAS, including the PFAS in EU's drinking water directive and a few other PFAS groups expected to be found in water (FTS; fluorotelomer sulfonic acids and PFASA; perfluoroalkane sulfonamides). Both source water (n=94) and finished drinking water (n=70) were analysed. The surface waters had a wide geographical spread east-west-south, but not northern part. Information about the technology used for treating the water was categorised (3 categories) to calculate removal efficiency (based on PFAS detected above 2xLOQ in source water). PFOS and brPFOS had the highest detection frequencies (95% in surface water and 89% and 94% in drinking water). Other PFAS that were frequently detected included PFBS and short PFCA (perfluorinated carboxylic acids, PFBA to PFNA) and PFHxS. Five PFAS were only detected in one drinking water source (PFBA, PFPS, PFHpS, N-EtFOSAA and PFHxSA). 15 of the measured PFAS were not detected above the LOQ in any samples representing mainly long PFCA and PFSA (perfluorosulfonic acids). The median number of PFAS in drinking water was 3, but up to 14 PFAS were detected in one sample. The lower bound sum of 20 PFAS included in EU's drinking water directive, was 0.28 ng/L (source water), while the maximum was 23 ng/L. For drinking water, the median was 0.26 ng/L and the maximum 12 ng/L. Denmark has regulated drinking water with a limit for the sum of 4 PFAS, risk assessed by EFSA, to 2 ng/L. Only one waterworks in Norway had levels above this limit in drinking water. The treatment efficiency at the waterworks were calculated based on PFAS concentrations in source water and drinking water taken the same day. The removal efficiency of PFAS for treatments with a combination of coagulation, dissolved air flotation, filtration and granulated activated carbon performed better for removing PFAS (ca. 60% removal of PFOS and brPFOS) than other categories. Details of the removal efficiency for different treatment stages at one treatment facility will be presented.

3.16 Poly- and perfluoroalkyl substances (PFASs): Addressing Urgent Questions in the 21st Century (Virtual Only)

3.16.V-01 Application of Standard Fate and Transport Modeling Approaches for Evaluating PFAS Compounds in Groundwater

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Per- and polyfluoroalkyl substances (PFAS) used in numerous consumer products and industrial applications make their way into the environment through multiple pathways. Vastness of the chemical group, with thousands of distinct PFAS and their widespread uses have led to their ubiquitous occurrence in the environment. These contaminants are receiving increased attention due to their persistence and toxicity to environmental and human health. Several small and large-scale monitoring programs have shown widespread presence of these compounds in air, surface and ground water, and soil media. USEPA has been prioritizing ongoing work to better understand and eventually reduce the potential exposure and risks caused by these chemicals. Reliable modeling tools to evaluate the fate and transport of these chemicals are critical to the development of risk assessment and remediation strategies. To date, limited work has been done to better understand the fate and transport of these complex chemicals in the environment using existing modeling approaches. Rising public interest and increasing regulatory action has made the need for modeling an important next step in advancing the understanding of these persistent chemicals. This work will focus on applying standard modeling approaches to understand the fate and transport of PFAS. Two models, HYDRUS and PEARL, were used to simulate PFAS measured under standardized conditions. As a case study, these models were then applied to simulate a PFAS chemical to two study areas in the United States. Results from modeling were compared with available groundwater monitoring data for the study areas. The practical utility of the standard modeling approaches for application to address the PFAS challenges over small and large geographies are discussed.

3.16.V-02 Multimedia Fate and Intake Fractions of PFAS Precursors and Terminal Degradation Products

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Here we present a database of physicochemical properties for over 70 PFAS, yield data for seven precursors in air, soil, freshwater, and freshwater sediment, and present an easily adaptable method to derive intake fractions of both precursors and terminal degradation products from multimedia transport throughout the environment. The database contains prioritized

experimental and estimated data, and supplies the critical input parameters needed to run a USEtox®-based model, which allows for the comparison of fate factors and intake fractions among different families of PFAS, and by chain length. This also provides a path for identifying the driving parameters of fate and transport for different PFAS. We use this data to calculate the total intake fractions by inhalation and ingestion associated with seven precursors (fluorotelomer alcohols and sulfonates) emitted to different compartments; each precursor's emission resulted in different intake profiles of terminal degradation products up to 0.01 kg.product.intake/kg.precursor.emitted. The total intake of terminal degradation products is more than intake of the initial parent compound in all cases. These results demonstrate the importance of considering multimedia transport of precursors, as they contribute significantly to direct exposure to PFAS. Future research should prioritize intermedia studies of fluorotelomer alcohols and sulfonates, and other precursors. Future risk assessments should consider multimedia transport and terminal degradation product exposure. Further toxicological studies on mixtures are needed as this work shows that individual precursor emission will result in the intake of multiple terminal degradation products.

3.16 Poly- and perfluoroalkyl substances (PFASs): Addressing Urgent Questions in the 21st Century (Poster)

3.16.P-Th110 What Happened to the Molecules? - Results and Molar Balances of 6: 2 and 8: 2 Polyfluoroalkyl Phosphate Diesters (diPAPs) After a Two-Year Soil Column Study Raise Questions

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PFAS (per- and polyfluorinated alkyl substances) have been the subject of continued environmental concerns for decades. Due to the ubiquitous occurrence of these anthropogenic “forever chemicals,” they represent a problem that must be dealt with on a global level. PFAAs (perfluoroalkyl acids), a group of PFAS, of which some compounds are already declared as PBT (persistent, bioaccumulative, toxic), are the final transformation products of transformable PFAS, called precursors. Polyfluoroalkyl phosphate esters (PAP), a group of precursors with different variations (e.g., diPAP), are found in paper industry products and thus also in agricultural lands after application of contaminated paper sludge polluted composts. Here, the first study on the simultaneous leaching and transformation behavior of diPAPs in soils is described, which may provide insights into possible cases of real-world contamination. Therefore, two representatives of diPAPs, 6:2 and 8:2 diPAP, were studied in 2-year soil column experiments, with a weekly water input of 35 ml, a biweekly analysis of eluate and a soil analysis at the end of study. 1.47 μmol and 1.17 μmol for 6:2 diPAP and 8:2 diPAP, respectively, were mixed into the topsoils. Due to the properties of diPAPs, a maximum conversion of 1 mol diPAPs equals 2 mol of PFAAs was taken as the base of molar balance calculations. After two years, the following molar amounts of PFAAs related to the initial molar amount of 6:2 diPAP were found in the leaching water: PFPeA (26.0 mol-%) > PFHxA (11.8 mol-%) > PFBA (4.0 mol-%) > PFHpA (0.1 mol-%). In the 8:2 diPAP study the major transformation product in leaching water was PFOA (16.5 mol-%) followed by PFHpA (3.2 mol-%), PFHxA (1.1 mol-%), PFPeA (0.9 mol-%) and PFBA (0.6 mol-%). 6.1 mol-% of 6:2 diPAP and 44.7 mol-% of 8:2 diPAP were still found in the topsoil layers at the end of the experiment, which shows a solely leaching potential of transformation products and a high sorption behaviour of both diPAPs. Small quantities of PFAAs in soil (6:2 diPAP: 0.9 mol %, 8:2 diPAP: 1.4 mol-%) cannot account for missing quantities in molar balances (6:2 diPAP: 51.0 mol-%, 8:2 diPAP: 31.6 mol-%). This loss may be related to the emergence of NERs (non-extractable residues), volatilisation of intermediates, not-quantified intermediates and/or loss through sorption on vessels. In other studies, missing/declining amounts were also explained by NERs, but the process by which this occurs remains unknown.

3.16.P-Th111 Analysing the Alternatives to Poly- and Perfluoroalkyl Substances Using Multicriteria Decision Analysis and Applying the Essential-Use Concept: A Fluorosurfactant Case Study

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Poly- and perfluoroalkyl substances (PFASs) are persistent, problematic, but often useful. European regulators plan to restrict PFASs and phase out uses not deemed essential for society. However, there are few case studies of the application of essential use concept in the literature. Analyses of the available alternatives must be carried out to identify uses that could be considered “substitutable” and to avoid regrettable substitution. In this case study alternatives to PFAS in coatings, paints and varnishes were investigated using multicriteria decision analysis (MCDA). The outcome of this was then applied to determine the essentiality of PFAS for this use. An established fluorosurfactant, 2 novel shorter-chain PFASs, and 2 nonfluorinated alternatives were identified. Data were gathered from the literature and in-silico tools for the substances and their anticipated environmental degradation products. These data were used to evaluate the performance of each alternative for each attribute contributing to objectives of low environmental hazard, low human health hazard, high functionality, and low cost. Weights for these attributes were derived to reflect the priorities of different stakeholders and the scores aggregated. Once the alternatives had been analysed the essentiality of PFAS for this use was determined. This case study demonstrates one possible application of the essential-use concept and contributes to the ongoing discussion surrounding this concept.

3.16.P-Th112 Implications of Results From a Multi-Generational Zebrafish PFOS Exposure-Response Study on Regulatory Thresholds for Protection of Aquatic Life

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The Environmental Protection Authority (EPA) of Victoria, Australia released water quality guidelines in 2016 for PFOS that included a freshwater 99% species protection level of 0.00023 µg/L, a values more than 100X to 1000X lower than screening values put forth by other regulatory agencies. Derivation of this exceedingly low threshold was driven largely, if not almost entirely, by results from a single study (Keiter et al. 2012), a multi-generational exposure of the zebrafish (*Danio rerio*) to PFOS, which reported reduced growth at 0.6 µg/L. Arblaster et al. (2017) previously described the limitations of the Keiter et al. (2012) study and how the 0.6 µg/L threshold, a value more than 10-fold lower than the next lowest toxicity value, affected the species sensitivity distribution used to derive the Australian WQ guideline. Despite the limitations of the Keiter et al. (2012) study design (e.g., low replication, small and unevenly spaced exposure range, and limited exposure verification), the reported result obviously had a significant influence on the Australian guidance and has the potential to influence future regulatory threshold values. Consequently, the USACE ERDC in collaboration with the USEPA and with support from SERDP, a coalition of businesses, conducted a multi-generational study of the effects of PFOS exposure on zebrafish using a more robust experimental design (e.g., 6 exposure concentrations [0.1, 0.6, 3.2, 20 & 100 ppb], 5 replicates, exposure concentration verification/validation) and an expanded suite of test endpoints (vitellogenin, RNA seq, histopathology). Based on results from this study involving 180-day exposures of both the parental (P1) and first familial (F1) generations, the strongest evidence of potential ecologically significant effects on zebrafish occurred at the 100 µg/L exposure concentration (i.e., the highest level evaluated) which showed potential effects on survival and growth, but no effects on reproduction. Results from this study provide strong evidence supporting an adverse effect threshold of 100 µg/L rather than the 0.6 µg/L (as reported by Keiter et al. 2012). The implications of these results are very significant, because the 100 µg/L threshold concentration translates to a 99% protection value of 1.3 µg/L rather than 0.00023 µg/L (as put forward by the EPA of Victoria, Australia). This presentation will describe and report the results from the USACE ERDC study and emphasize the importance of the technical basis for development of credible threshold concentrations.

3.16.P-Th113 Development and Demonstration of Volatile and Semi-Volatile Per- and Polyfluoroalkyl Substances (PFAS) GC-MS Methods on Select NIST Reference Materials

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Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants of concern, and have been observed in consumer products, consumer byproducts (e.g., dust, wastewater, and landfill leachate), and environmental compartments including humans and biota. As a result, routine monitoring methods, method development, and commercial analysis of these compounds have become more widely available. Current commercial methods typically target ionic, less volatile PFAS by liquid chromatography tandem mass spectrometry (LC-MS/MS). However, volatile and semi-volatile analytes are not commonly included despite the fact that volatile and ionic PFAS are known to co-occur as a result of manufacturing processes. Further, volatile PFAS are commonly excluded due to their poor sensitivity by LC-MS/MS and a lack of materials available to validate analytical methods (e.g., Standard Reference Materials (SRMs) and analytical standards). To fill this data gap, multiple instrumental methods for twelve volatile and semi-volatile PFAS including 4:2, 5:2s, 6:2, 7:2s, 8:2, and 10:2 fluorotelomer alcohols, 8:2 and 10:2 perfluoroalkyl acrylates and perfluoroalkyl acetates, and methyl- and ethylperfluorooctanesulfonamide, have been developed for electrospray ionization gas-chromatography mass spectrometry (EI GC-MS). To demonstrate the efficacy of the methodology two Standard Reference Materials (SRMs) from NIST, SRM 2585 Organic Contaminants in House Dust and SRM 2781 Domestic Sludge were selected for quantitative evaluation of GC amenable PFAS. Each of these materials represents a different analytical compartment, and both materials have reported concentrations of ionic PFAS by LC-MS/MS on their Certificates of Analysis. The observed volatile PFAS concentrations in the selected SRMs are subsequently evaluated across instrumentation (two separate GC platforms) and across acquisition modes to evaluate accuracy and precision. These volatile PFAS measurements add value to the volatile PFAS knowledge-base and provide needed methodological, analytical validation materials.

3.16.P-Th114 Modelling the Environmental Distribution of GenX: From Point Source to Remote Areas

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Per- and poly fluoroalkyl substances (PFAS) are a group of chemicals that includes several thousand fluorinated substances. PFAS are known for their persistent nature once released into the environment and several PFAS possess chemical properties which makes them mobile in the aquatic environment. A particular concern for these types of substances is that they may have the potential to travel to areas far from the point of release and eventually reach concentrations that could result in adverse environmental effects. To investigate the travel potential of mobile PFAS, GenX [hexafluoropropylene oxide dimer acid (HFPO-DA)] was selected as an example PFAS. Data on chemical properties used for the modelling were obtained from open access sources. There is one known fluorochemical production plant in Europe (in the Netherlands) which uses GenX in the production of fluoropolymers and data on tonnages and/or releases to water and/or air between 2012 and 2019 were reviewed. Predicted environmental concentrations (PECs) were estimated at local, regional and continental scales using the EUSES model as well as the Multimedia Stock Pollution Tool (MUST) which allows for time-dependent emission scenarios. Based on emission data, PECs

for surface water at the local scale ranged from 3 µg/L in 2012 up to around 8 µg/L in 2017. For 2019, when emissions to water were reduced, the local PEC was estimated at about 0.007 µg/L. Existing monitoring data measured downstream of the GenX processing site were generally a factor of around 10 lower than the local PECs. Freshwater PECs followed the emission pattern with the highest regional and continental concentrations estimated in 2017 at 0.026 µg/L and 0.0025 µg/L respectively. These PECs are generally higher than monitoring data from surface water samples collected in the Netherlands and elsewhere in Europe. Further modelling showed that when emissions are ceased, water PECs at the regional scale decrease rapidly within 1 year. However, they will take up to 2 years to decrease to near zero at the continental scale. The modelling suggests that local and regional concentrations of GenX are expected to respond quickly to changes in emission. Furthermore, in order to reach surface water concentrations that would surpass the water quality limit set by national authorities, the annual amount released into the regional scale would have to be >4 times higher than the highest reported emissions to water.

3.16.P-Th115 Assessing Human Health Risks From Per- and Polyfluoroalkyl Substances (PFAS) Via Drinking Water

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Per- and polyfluoroalkyl substances (PFAS) are a group of water-soluble chemical compounds that have a wide range of applications but are also very persistent and show hazardous properties. The European Food Safety Authority (EFSA) has concluded that drinking water and food are the main PFAS exposure routes for humans and recommends a safe level for the total weekly intake (TWI) of 4.4 ng/kg b.w. for the sum of four PFAS. This study investigates the human exposure levels to PFAS, including very polar ultrashort-chain (C1-C3) and branched isomers of PFAS, in drinking water and evaluates the human risk based on the EFSA TWI. An analytical method for determining trace levels of 56 targeted PFAS in drinking water (including the ultrashort-chain PFAS and branched isomers) was developed by using solid-phase extraction, chromatographic separation (UPLC CSH C18 column), and heated electrospray ionization (ESI). The method showed effective elimination of matrix effects produced by using heated ESI as an ion source in HRMS. The method was successfully applied to 18 drinking water samples in the Netherlands from different water sources (groundwater and surface water) and their corresponding raw water. The limit of detection (LOD) for targeted analytes was in the range of 9 pg/L-100 pg/L, except for TFA and PFPrA with a higher LOD of 35440 pg/L and 240 pg/L, respectively. The ultrashort-chain PFAS (0.09 µg/L - 1.1 µg/L) followed by the perfluoroalkyl carboxylic acids (PFCA) (5.2 ng/L - 79.5 ng/L) were the dominant classes. The analysis of variance for PFCA, perfluoroalkyl sulfonic acid (PFSA), and precursors showed statistically significant differences between drinking water originating from different sources. No statistically significant difference was found for the ultrashort-chain PFAS. The branched isomer contribution to the sum of linear and branched for PFOS (29% to 64%), and PFHxS (17% to 24%) were different from the original production ratio (20-30% branched for PFOS, and ~ 5% branched for PFHxS). Drinking water produced from surface water, except for two samples using a GAC filtration step in their treatment processes, exceeded the safe value derived from the EFSA-TWI. Further research is required to better assess the exposure to higher levels of ultrashort-chain PFAS and branched isomers present in drinking water. Extra efforts should be directed for protecting the groundwater reservoir from the very polar compounds like ultrashort-chain PFAS.

3.16.P-Th116 Suspect and Non-Target Screening Method Development for PFAS Using Lc-Hrms

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Per- and polyfluorinated substances (PFAS) are man-made chemicals which have unique lipophobic and hydrophobic properties, and thus has found much use in various products around the world for its non-stick properties. However, PFAS can pose a health risk and is bioaccumulative, which in turn makes it a persistent organic pollutant and with its unique chemical properties highly mobile. Currently there are over 4000 registered PFAS in multiple publicly available databases such as the US EPA, Norman Network, and the Swedish Chemical Agency, but still only a very small fraction of those are monitored in routine analyses. Furthermore, new destructive treatment techniques are being developed to break down PFASs. Little is known about the production of PFAS transformation products that may occur from these treatments. The research aims to develop a method that can perform both suspect screening and non-target screening of PFAS to elucidate the contaminants that are missed in common routine analyses. Liquid chromatography-high-resolution mass spectrometry will be utilized for the development of these screening methods. With the high-resolution mass spectrometry the accurate masses obtained can be compared to online databases for identifying suspects. The suspects can then be further identified with MS² experiments and with authentic standards. For the non-target screening, novel software approaches will be utilized to identify new PFASs. Appropriate mass spectrometry methods such as Data-Dependent Analysis (DDA), and Data-Independent Analysis (DIA) will be utilized to obtain as much information as possible of the samples. The final results will elucidate the presence of previously known PFASs and newly created PFAS transformation products that may occur from destructive treatments.

3.16.P-Th117 PFAS in Recycled Fertilisers: Potential Issues, Identified Uncertainties, and Need for Monitoring

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The findings presented in this publication resulted from an assessment which was performed in view of a project for the European Commission, Directorate General Environment. In this project, the exposure and risk of contaminants/impurities of potential concern in fertilisers needed to be assessed, to identify contaminants/impurities or fertilisers for which additional regulatory measures may be required. PFAS were identified as one of the (groups of) contaminants for which an assessment was required.

The focus was on the assessment of fertilisers based on or containing recycled materials derived from sewage sludge. PFOA and PFHxA were assessed as representative for the long-chain and short-chain PFAS, respectively. As one of the major data gaps identified was evidence on the removal efficiency of recycling processes, an indicative ‘screening’ assessment was performed assuming a concentration of PFOA and PFHxA equal to an existing national concentration limit for fertilisers. It should be noted that the outcome of the assessment, in which predicted exposure is compared to Environmental Quality Standards and Tolerable Daily/Weekly Intakes, is to be considered as ‘indicative’, as the obtained ratios are not to be interpreted as true risk quotients for substances following a ‘non-threshold’ approach. As exceedances of established standards have been calculated for PFOA as representative of long-chain PFAS, mainly for secondary poisoning and humans exposed via the environment, it became clear that it is of utmost importance to verify that recycling processes of which the output materials are used in/as fertilisers can guarantee a very high removal efficiency. If this cannot be guaranteed, further build-up of PFAS in the environment due to increasing use of recycled fertilisers could not be avoided. It should be noted that the views expressed in the poster are those of the contractor with the context of the service contract 070201/2019/817112/SER/ENV.B2 and according to the terms of reference associated with that contract.

3.16.P-Th118 Current Gaps and Research Needs of Adsorption Process As the BEST Available Treatment Technology for PFAS Removal From WATER

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Per- and polyfluoroalkyl substances (PFAS) are a large group of anthropogenic compounds that received particular attention due to their persistence and toxicity. The recalcitrant behaviour of PFAS along with their chemical properties nicknames them as “forever chemicals” and poses challenges to conventional water and wastewater treatment processes. Adsorption process is the most extensively implemented technology for PFAS removal. PFAS adsorption onto granular activated carbon (GAC) or anion exchange resin (AER) is a field-proven technology. However, several limitations of prior researches dealing with adsorption processes can be highlighted. Available literature reveals that the adsorption efficiency depends on PFAS chain length and functional group. The adsorption mechanisms of short- and long-chain PFAS are different due to their different structure and hydrophobicity. Short-chain PFAS are more hydrophilic and tend to be less adsorbed than long-chain ones. While, at the same chain length, perfluoroalkyl sulfonic acids (PFSAs) are removed better than perfluoroalkyl carboxylic acids (PFCAs). Adsorption capacity also depends on adsorbents properties. The acrylic resins are more effective for PFAS removal than styrenic resins, while macroporous resins exhibit better uptake than gel resins. Similarly, macro- and meso-porous GAC exhibited better long-chain PFAS removal. Despite its properties are similar to AC, biochar exhibits lower adsorption capacity than AC. During adsorption processes, organic matter, specifically natural organic matter (NOM), could interact with PFAS through electrostatic and/or hydrophobic interactions, affecting the adsorption efficiency of adsorbent materials. Despite the widespread application, the main drawback and limitation is currently the regeneration of PFAS-saturated adsorbents which is essential to renew the adsorption capacity and to recover adsorbate PFAS. Conversely, a relevant knowledge gap regarding thermal regeneration techniques for PFAS-saturated GAC should be highlighted. To sum up, AERs are more effective than AC for both long- and short-chain PFAS removal, while the adsorption capacity of short-chain PFAS is lower than that observed for long-chain ones. Extensive research efforts should be routed to the investigation of effective regeneration techniques. Consequently, economic assessments should also address adsorbents disposal and/or regeneration.

3.16.P-Th119 Sorption of PFAS to Activated and Non-Activated Waste Biochars for In Situ Remediation of Pfas-Contaminated Soil and WATER

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Due to considerable persistence, bioaccumulation, and toxicity of per- and polyfluoroalkyl substances (PFASs), remediation of soil and water contaminated with PFAS is urgently needed. The potential for using various organic waste materials in the production of biochar sorbents for PFAS has sparked great interest among researchers. In a recent study by Sørmo et al. (2021), eight activated waste timber biochars applied with a 5% amendment dose to heavily PFAS-contaminated, low-TOC (1.6%) soil, reduced leachate concentration with 98-100%. Since strong sorption was seen for these waste timber biochars, the present work tested the sorption behavior of two biochars from sewage sludge pyrolyzed at 700 °C to C4-9 perfluorinated carboxylic acids (PFCAs). The sludge feedstocks were 1) Ullensaker sludge (ULS) containing PFAS-enriched wastewater from Oslo Airport, and 2) Biorest Lindum (BRL), a sludge digestate. In laboratory batch experiments, we prepared ten-point biochar-water sorption isotherms with ULS and BRL and six-point soil-biochar-water isotherms using a clean, sandy soil (1.3% TOC) and biochar at 2% w/w spiked with both a PFCA cocktail and C4-9 individually. Previous studies of pyrolyzed sewage sludge conclude with poor sorption to PFAS. However, a preliminary analysis of a selection of ULS batch samples (n=22/178) indicate sorption almost as strong to ULS as clean wood chips, the experimental control. The initial spike concentrations were reduced with >98% for all PFCAs tested, where K_d ranged from 4.29-5.85 by increasing chain length in accordance with the literature. Even though elemental composition analysis of ULS is yet to be conducted, sludge char is expected to have low carbon content, porosity, and internal surface area—properties associated with poor sorption—and instead be enriched with minerals, especially iron oxides. Since ULS sorbs stronger than expected, we speculate that the sorption mechanisms between this biochar and PFAS is not dominated by hydrophobic interactions that were observed for waste timber in Sørmo et al., but by electrostatic forces between mineral phases in the sludge matrix and the carboxylate groups of PFCAs. The high sorption capacity of ULS is promising for the implementation of a sustainable and cost-effective waste management method of problematic wastes such as contaminated sewage sludge, and will be an important contributor to a circular economy. Note: Complete results will be presented in May 2022.

3.16.P-Th120 Short-Chain Per- and Polyfluoroalkyl Substances (PFAS) Effects on Human Phase I Biotransformation Enzymes

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Per- and polyfluoroalkyl substances (PFAS) are a class of compounds that have been of interest to human and ecological risk assessors in recent years because they are found ubiquitously and there is evidence for adverse health effects in humans. Newer, short-chain alternatives have been developed to mitigate some of the toxic effects observed with the long-chain variants. However, there is a significant lack of toxicity data pertaining to these compounds, and even less is known about their effects in the context of exposures with other xenobiotics. A previous study has suggested that two of the legacy PFAS, perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), are able to reduce the expression of important CYP-enzymes in human liver cells following exposure. We evaluated the activity of two phase I biotransformation pathways (CYP1A1 and CYP3A4) following exposure to short-chain PFAS, including perfluorobutane sulfonic acid (PFBS), 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate (GenX), perfluorohexane sulfonate (PFHxS), perfluorohexanoic acid (PFHxA), 6:2 fluorotelomer alcohol (6:2 FTOH), using a differentiated human liver cell line (HepaRG). In addition to the cell lines, the inhibitory potential of the long-chain and short-chain PFAS was also evaluated in human CYP1A1, -1A2, -2C19, -2D6, -2E1, and 2B6 supersomes. Following single exposures, each of the compounds was co-exposed to known CYP enzyme inducers, Rifampicin and Benzo(a)pyrene, to evaluate the inhibition potential of the short-chain PFAS. Our results indicate that the interference of short-chain PFAS with CYP1A1 and CYP3A4 enzymes could potentially lead to adverse outcomes resulting from the inability of biotransformation pathways to function as needed.

3.16.P-Th121 An Analysis of How the Current REACH Processes May Serve As a Basis for Implementing the "Essential-Use" Concept for PFASs

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Per- and polyfluoroalkyl substances (PFASs) are a class of synthetic organic substances with diverse structures, properties, uses, bioaccumulation potentials, environmental mobilities and toxicities. Despite this high diversity, all PFASs are alike in that they contain perfluoroalkyl moieties that are extremely resistant to environmental and metabolic degradation. The vast majority of PFASs are therefore either non-degradable or transform ultimately into stable terminal transformation products (which are still PFASs). Many have argued that this high persistence is sufficient concern for their management as a chemical class, for all "non-essential" uses of PFASs to be phased out, and for fostering research, innovation and transition to safer alternatives in the case of uses that currently are essential. Currently, five European Union (EU) Member States have announced that they are preparing a wide restriction proposal for the entire class of PFASs under the EU Chemicals Regulation--REACH. This study aims to shed light on what has generally been established under REACH with relevance to the "essential-use" concept. In particular, it analyzes which argumentations have been used to conclude on individual exemptions in the current REACH restriction process. This analysis is then used to evaluate whether and how future application of the "essential-use" concept under REACH could be possible, also for improving the legislation.

3.16.P-Th122 The New Definition of PFAS - Too Long to Be True?

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The recently proposed definition of PFAS - Per- and Polyfluoroalkyl Substances - reads: "PFASs are defined as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e., with a few noted exceptions, any chemical with at least a perfluorinated methyl group (?CF₃) or a perfluorinated methylene group (?CF₂?) is a PFAS". With this broad definition the PFAS universe includes too many substances that are too far from the original meaning of the term PFAS; at the same time it inexcusably excludes a number of really dangerous fluorinated chemicals. First, the definition can be shortened to: PFASs are chemicals with a non-terminal CF₂ group, not connected to H, Cl, Br or I. Indeed, each CF₃-group is nothing, but a CF₂-group connected to F, and, therefore, there is no need to mention both. There is no justification for exclusion of CF₂-groups connected to H, Cl, Br or I and terminal ones. Indeed, CFC-113 and CFC-113a, HFC-134 and HFC-134a must fall into the same group. Removal of "noted exceptions" will shorten the definition to: PFAS are chemicals with CF₂ group. The laconic four-words definition - chemicals with CF₂ group - will be easy to use by non-experts. The romantic title, "forever chemicals", reflects the spirit of PFAS - extreme persistence due to effect of polyfluorination. This must be reflected in the definition of PFAS. Then PFAS - the term already in our hearts - can be redefined as Persistent FluoroAliphatic Substances. All non-aromatic substances are aliphatic and include alkyl, cycloalkyl, bicycloalkyl and alkylene derivatives. All persistent substances of OECD definition will be included, and a few other important ones. Non-persistent substances of OECD definition could be classified as Precursors of PFAS (PPFASs) or Potential Precursors of PFAS (PPPFASs). Yes, it will probably take a scientist and not a software to relate a formula to PFAS or not.

3.17 Qualitative and quantitative methodologies for the biomonitoring of environmental organic pollutants and their metabolites

3.17.T-01 Development and Optimization of an Extraction Method Based on Use/D-Spe for the Analysis of Pollutants of Emerging Concern in Invasive and Native Aquatic Biota From l'Albufera Natural Park (Spain)

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Exotic species such as *Procambarus clarkii*, *Corbicula fluminea*, and *Leopomis gibbosus* have become invasive species in Spain for some decades because they have managed to adapt in Mediterranean wetlands, including the Natural Park of L'Albufera (Valencia). This kind of species, as well as native fauna such as *Anodonta sp.*, may be exposed to a wide range of emerging contaminants and they could be used as bioindicators to assess chemical exposure and pressure on freshwater ecosystem species. In this study, we developed and optimized an extraction method assisted by ultrasound and d-SPE for the simultaneous determination and quantification of different pollutants of emerging concern including 91 pharmaceuticals (PhACs), 11 flame retardants, 21 PFAs, and 55 pesticides. For this purpose, four extraction solvents (1) ACN:iPrOH (3:1) + 0.1%, (2) ACN + 0.1% F.A, (3) MeOH/H₂O (7:3) + 1% F.A (4) MeOH and three cleaning sorbents (1) Z-sep, (2) Z-sep+ (3) Z-sep/C18 were tested. Recovery efficiency tests were performed for each of them and the solvent and sorbent with which the best results were obtained were validated in two of the matrices (*Procambarus clarkii* and *Anodonta sp.*). To demonstrate the effectiveness of the method, wild samples of the invasive species were collected from L'Albufera Natural Park. Preliminary results indicate the presence of some PhACs such as sulfamethoxazole, citalopram, cotinine, tramadol, and methadone.

3.17.T-02 Chemometers: An Integrative Tool for Studying Chemicals in Biota and Surrounding Abiotic Media

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Chemometers are a common and well-defined polymer reference phase for passive equilibrium sampling of a large range of organic pollutants in different matrices like biota, sediment and water. By bringing the *chemometer* into direct contact with the sample, the chemicals partition between the sample and the polymer until a thermodynamic equilibrium is established. As the *chemometers* are defined as a common reference phase, the chemical concentrations in the *chemometers* at equilibrium can be directly compared between different biotic and abiotic compartments and organisms of different trophic levels. This approach has opened a new analytical window for determining the chemical activity of pollutants, which is one of the main drivers for partitioning, biouptake and toxicity. It allows to express the data on a common basis, as the equilibrium partitioning concentrations in the silicone, circumventing normalization, and the extracts of the *chemometers* can both be submitted to chemical analysis and/or to bioanalytical profiling. The *chemometers* have proven to be a relevant tool to evaluate chemical pollutants in biota, and we are currently applying them in diverse applications, amongst others to study (I) the thermodynamics of bioaccumulation in aquatic environments using multimedia sampling in a remote Swedish lake, (II) the internal exposure in marine mammal tissues from the German North and Baltic Sea coasts, and (III) passive sampling in the abiotic compartments sediment and water from German rivers with different patterns and levels of pollution as a proxy for chemical concentrations in biota. This presentation will give an overview of our related activities and applications of *chemometers* aimed at characterizing the complex environmental exposure by mixtures of pollutants at high trophic levels using a common reference phase. An outlook will cover future plans for applications to describe the lifelong exposure to mixtures of pollutants, the so-called exposome, of top predators.

3.17.T-03 FR Exposure Assessment of E-Waste Dismantlers Using Different Sampling Strategies

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Nowadays electronic waste (e-waste) is the fastest growing waste on the planet. In 2019, 54 million tonnes were generated in the world, while only a 17% of them were recycled. A growth to 75 million tonnes is expected for 2030. E-waste is usually made of plastic, ceramics, glass or metal, that are valuable and reusable materials when they are properly recycled, which results into an increase of the e-waste recycling industry. Potentially toxic substances as flame retardants (FRs) can be released to the air during recycling process, which can suppose a risk for the employees. Sampling was conducted in an e-waste dismantling facility from Catalonia (Spain), where a wide range of products were recycled. Facility was divided in two floors that were sampled separately, grinding floor and cathode-ray tube (CRT) dismantling floor. Employees wore cotton t-shirts and polydimethylsiloxane wristbands during workday and they returned it once finished. Airborne fine particulate matter (PM_{2.5}) was also collected. Organophosphate esters (OPEs) and halogenated flame retardants (HFRs), specifically polybrominated diphenyl ethers (PBDEs), novel brominated FRs (NBFRs) and dechloranes, were analysed in all samples. OPEs by TFC-LC-MS-MS and HFRs by GC-MS-MS. OPEs, PBDEs and NBFRs were detected in all samples, but dechloranes were not detected in PM_{2.5} samples from grinding area neither in one from CRT area. PM_{2.5} samples presented higher concentrations of FRs in CRT area compared with grinding area. Concentration levels detected in PM_{2.5} were higher than those reported from other studies in Australian population, but similar to the ones reported from an e-waste dismantling facility from Canada. Results from wristbands show also the exposure difference between areas, as in PM_{2.5}. Comparing profiles of both sampling methods there were found some differences. Percentage contribution to the OPEs was 3% for DCP in PM_{2.5}, while it increased to 36% in wristbands, being the most contributing OPE in this matrix. For PBDEs, the second compound with highest contribution was different in each sampling method, being BDE-183 in PM_{2.5} and BDE-99 in wristbands. Dechloranes were detected in all wristband samples, but only in two samples of PM_{2.5}. NBFRs presented similar profiles in both sampling methods.

3.17.T-04 Comparison of Sample Treatment Procedures for Human Exposome Evaluation in Urine Through Suspect Screening

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The concept of exposome has gained popularity in recent years due to the constant exposure to chemical compounds that humans face. Those compounds can be found everywhere, from personal care products (PCPs) to plastic materials or biocides, and can easily be introduced to our organisms. Within our bodies, xenobiotics are usually metabolized (mostly as glucuronides) with the aim of increasing their polarity in order to be excreted through urine. This is why urine has become the most relevant biofluid for exposome monitorization in humans. Most works in the literature are limited to selected analytes, instead of analyzing as many compounds as possible. Therefore, in this work, several procedures (solid phase extraction, liquid-liquid extraction and “dilute and shoot” (SPE, LLE and DS)) have been tested not only for target analysis of a wide variety of analytes (230 targets), but also for identifying other suspects (more than 4000) and glucuronides by means of LC-HRMS. Synthetic urine samples were spiked at 5, 15 and 30 ng g⁻¹ with the target analytes and absolute recoveries were calculated at each spiking-level. Although best recoveries were obtained with LLE overall, most phthalate metabolites were not recovered with that technique, which is a relevant exposome biomarker family. With SPE instead, all families were detected even though lower recoveries were achieved. However, with surrogate correction, apparent recoveries were statistically comparable. Lastly, DS showed the worst results at low spiking level due to the lack of preconcentration and high matrix effect at the detection. Regarding the real urine samples, they were treated with and without enzymatic hydrolysis for glucuronide deconjugation and several xenobiotics were quantified in the 3 volunteers at ng g⁻¹ level. Moreover, suspect screening was performed for detecting other exposome related compounds besides the ones included in the target analysis method. In this case, samples treated with SPE differed from LLE and DS independently of the volunteer, due to the considerably higher number of xenobiotics identified with SPE. A similar tendency was observed for the non-target analysis of glucuronides, where many more glucuronides were detected with SPE.

3.17.T-05 New Methods for Confirmation of Pesticide Metabolites in Human Biomonitoring

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Chemicals of emerging concern include a wide range of different chemical groups, among them pesticides, which are widely used in modern agriculture or as ingredients of biocidal products. Human biomonitoring (HBM) assesses the internal exposure to pesticides, with urine being the most common matrix analyzed. HBM of pesticides is challenging because of the high number of pesticides, the fact that in most cases they are not excreted as such, but as many possible or even unknown metabolites, and the unavailability of analytical reference standards of the metabolites. This calls for qualitative HRMS-based screening methods to assess overall exposure. The result of such screening methods are mostly tentative detects which require a follow-up confirmatory analysis. We present new methods for confirmation of pesticide metabolites in human urine by applying liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) and computational data analysis tools. As proof of the concept for our methods, we exemplify the application on the urine samples of the large-scale SPECIMEn (Survey on Pesticide Mixtures in Europe) study within the HBM4EU project (www.hbm4eu.eu). For the confirmation of *phase I metabolites* (mainly formed by CYP-450 mediated oxidative reactions), we present a high-throughput strategy by generating phase I metabolites *in-vitro* by incubation of the pesticides with highly pooled human liver S9 extract. The extracted retention time and the reference tandem mass spectra are matched with spectra received from representative urine samples. For the confirmation of *phase II metabolites* (formed by conjugation reactions), we studied the comparability of the tandem mass spectra of the conjugated metabolite with the unconjugated species. Based on our observations on glucuronidated drugs in a clinical dataset, we developed a strategy to modify the tandem mass spectra of conjugates to compare them with reference spectra of the deconjugated species. In total, we confirmed eight parent pesticides and more than 30 pesticides metabolites (confidence level 1, 2a/b according to Schymanski et al.) in the dataset of the SPECIMEn study. This demonstrates the applicability of the described method for the confirmation of pesticide metabolites, increasing the confidence of tentative annotations. The obtained list of pesticide metabolites will help in further quantitative studies to select possible biomarkers of exposure. As expected, we detected more metabolites than parent pesticides, which highlights the need for novel approaches to overcome limited standard availability and also include more pesticide metabolites in HBM studies.

3.17 Qualitative and quantitative methodologies for the biomonitoring of environmental organic pollutants and their metabolites (Poster)

3.17.P-Tu181 Constructed Wetlands As an Efficient Technology to Remove Organic Contaminants From Municipal Treated Wastewater

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Ensuring water quality and safety is one of the most important environmental challenges of this century. To improve and reuse water, wastewater treatment plants (WWTPs) have been implemented to reduce the pollution of industrial and urban wastewater through different treatments and processes. However, WWTPs are identified as a major source of organic contaminants (OCs) in aquatic environments because they cannot remove them. The occurrence of OCs in aquatic environments could pose a risk to human health and environment. Thus, specific treatments are needed to remove these OCs and preserve the environment. Although some processes have been tested, research on nature-based treatments to that proposed are limited. In this study, four constructed wetlands (CW) were assembled at semi-pilot scale in vertical CW with recirculation. Each one was filled with 30 L of spiked secondary wastewater from the WWTP located in Barcelona (Spain). Each microcosm was made using methacrylate box (L: 100 cm, W: 30 cm, H: 30 cm) filled with first layer of cobbles, a second layer of volcanic rocks, a third layer of fine gravel and, finally, a layer of sand. In three of them, an additional layer of burnt cork, almond shell or chestnut shell was added. These wastes present high adsorption properties, which could help improve the efficiency of CW promoting the circular economy. The last one, without any adsorbent, was used as control. The concentration of the target MPs was analysed using high-resolution mass spectrometry Q-Exactive Orbitrap. Suspended solids and dissolved organic carbon were also determined in each experiment. Finally, to determine the suitability of this technique for reuse in agriculture, the treated effluents will be tested to irrigate lettuce samples that will be subjected to analysis of residues. The preliminary results in the “control wetland” revealed that more than 80% of some OCs were removed at 4 days of retention time. However, the OCs with high log_{Kow} were eliminated less than 40% in the same system. For its part, the suspended solids were removed about 80%. **Acknowledgements:** This work was financially supported by Spanish national project TRAPPER (CICLIC, RTI2018-097158-B-C33) and “Agencia Estatal de Investigación” from the Spanish Ministry of Science and Innovation and the IDAEA-CSIC, a Centre of Excellence Severo Ochoa (CEX2018-000794-S). Núria López thanks to the Spanish Ministry of Universities and Universitat de Barcelona for Margarita Salas contract.

3.17.P-Tu182 Evaluation of Aromatic Amines Levels in Textile Products From Spain and Brazil

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Azo dyes are commonly used in the textile industry. These colorful compounds are insoluble in water and are synthesized into the fiber during the dyeing process. The fundamental composition of azo precursors contains one or more aromatic amine molecules (AAs), currently considered as emerging pollutants by regulatory agencies. It has been demonstrated that after exposure to textile products containing azo dyes, skin bacteria reduce azo dyes and then release AAs, eventually absorbed through skin. These compounds are polar organic chemicals that derive from aromatic hydrocarbons and show a widespread distribution. The International Agency for Research on Cancer (IARC) classified the AAs into three categories: known, probable, and possible human carcinogens. This study is aimed at determining the content of AAs in organic and non-organic clothes from Spain and Brazil. Fifty-eight aromatic amines, regulated and non-regulated, were included. Of them, twenty-two amines are known carcinogens and regulated under the European Union (REACH regulation; No. 1907/2006), such as 2-naphthylamine and 4-chloroaniline. Commercial samples bought in both Spain and Brazil were made of different fabric materials, including cotton, wool, silk, polyamide, acrylic, viscose, and polyester. The selected items were from pregnant women, newborns, and children, vulnerable groups of the populations to develop adverse effects after chemicals exposure. The sample preparation method was based on ISO DIN EN 14362-1 (Spanish Standard UNE-EN, September 2017), with adaptations. The instrumental analysis was performed employing high-performance liquid chromatography (HPLC) with an interface of Orbitrap Exploris™ 120 mass spectrometer (Thermo Scientific™), while the chromatographic separation was carried out using an Acquity UPLC BEH C18 (1.7 μm, 2.1 x 150 mm). The data of this project will be relevant to support public health federal/national agencies to establish the baseline values for aromatic amines in textile products.

3.17.P-Tu183 Advanced Throughput Analytical Strategies for the Comprehensive HRMS Screening of Organic Micropollutants in Marine Mammals From the Baltic Sea

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The systematic monitoring of organic micropollutants in the aquatic ecosystem has revealed the ubiquitous presence of numerous anthropogenic chemicals in different environmental compartments, such as water, sediments, and biota. Although there is established knowledge on the presence of regulated priority pollutants and their adverse effects on the aquatic organisms, monitoring data for emerging contaminants are still scarce. High Resolution Mass Spectrometry (HRMS) - based workflows appear to be a powerful tool for the simultaneous determination of organic micropollutants, having a wide range of uses, applications and physicochemical properties, as well as their (bio)transformation products. Consequently, there is an urgent need for sufficient occurrence data to be able to evaluate the emergence of these compounds and initiate risk mitigation measures. Biomonitoring studies using wildlife can be a valuable source of data for the assessment of the territorial and the temporal distribution of organic micropollutants. Furthermore, chemicals detected in organisms are possibly highly bio-accumulative (B) and persistent (P), thus fulfilling two out of three PBT criteria considered under REACH legislation. Therefore, the systematic biomonitoring of organic micropollutants will contribute to the assessment of the EU risk mitigation measures, but will also

provide insights into the emergence of these chemicals, the ecosystem condition, and the potential adverse effects on the human health. In the current biomonitoring survey, a holistic analytical approach was applied for the determination of organic micropollutants in marine mammal specimens, combining complementary chromatographic techniques and ionization modes coupled to HRMS (LC-ESI-QToF MS and GC-APCI-QToF MS). In this context, 11 liver samples from 4 different marine mammal species (common dolphin, harbour porpoise, harbour seal and grey seal), collected from the Baltic Sea, were analyzed following state-of-the-art analytical methodologies. Generic and validated analytical protocols were employed and the acquired HRMS chromatograms were screened for the presence of more than 2,400 known and 65,000 environmentally relevant suspect organic micropollutants following wide-scope target and suspect screening methodologies, respectively. Moreover, all HRMS data were digitally archived in the NORMAN Digital Sample Freezing Platform for further retrospective suspect screening.

3.17.P-Tu184 From Target to Suspect and Non-Target Screening of Endocrine Disrupting Compounds in Human Urine

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Synthetic organic compounds are useful for a wide variety of applications, since they are key components of personal care products, plastics or several biocides, among others. However, they get leached from those materials, becoming ubiquitous and posing a serious threat to humans' welfare. In this scenario, endocrine disrupting compounds (EDCs) have been in the spotlight in recent years, since they interfere with the endocrine system, leading to adverse health issues. Within our bodies, EDCs are metabolized in order to be excreted through urine. Therefore, urine has become the most important biofluid for exposome monitorization in humans. Regarding the quantification of EDCs in urine, liquid chromatography coupled to low resolution tandem mass spectrometry (LC-MS/MS) is mostly used through target analysis approaches. However, with the emerge of high resolution mass spectrometry (HRMS), new analysis possibilities are possible, such as, suspect and non-target screening (SNTS). Consequently, the identification of other compounds that are usually overlooked in most routine analysis is possible. At the present work, an analytical method based on the use of both LC-MS/MS and LC-HRMS/MS techniques for target and SNTS in human urine has been developed. Firstly, the target analysis method for 24 EDCs (including bisphenols, parabens, benzophenones, phthalates, triclosan and triclocarban) was optimized deeply, studying the enzymatic hydrolysis, clean-up by solid-phase extraction and analysis by LC-MS/MS. Then, the method was fully validated, obtaining satisfactory figures of merit and applied to volunteers' samples, in which several EDCs were quantified. For instance, benzophenone-3 ($0.2 - 13 \text{ ng g}^{-1}$), bisphenol A ($7.7 - 13.7 \text{ ng g}^{-1}$), methyl 3,5-dihydroxybenzoate ($8 - 254 \text{ ng g}^{-1}$), mono butyl phthalate ($2 - 17 \text{ ng g}^{-1}$) and triclosan ($0.3 - 9 \text{ ng g}^{-1}$) were found at higher concentrations than the rest of the analytes. Nevertheless, the presence of other analogues was detected as well. In addition, the target analysis method was extended to suspect analysis (almost 4000 suspects) and non-target analysis (molecules containing S, Cl or Br atoms) with the data obtained from LC-HRMS/MS. That strategy allowed detecting other 74 compounds (personal care products, plasticizers and exposome biomarkers) that would not be noticed in typical routine target analysis approaches due to the reduced number of target analytes.

3.17.P-Tu185 Active Bumblebees As Passive Samplers

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The decline of insect populations is gaining increased public attention, as the lack of key pollinators becomes a pressing issue for agriculture. Natural habitats for pollinators such as honey bees and bumblebees are being lost due to fragmentation, land-use changes, the use of fertilizers and the application of pesticides. This forces pollinators into urban surroundings, where they find small scale habitats in parks and green infrastructure. In urban, as well as agrarian landscapes, various anthropogenic activities lead to diverse pollutant emissions. In both habitats, pollinators are expected to be exposed to these pollutant profiles during their lifetime, e.g. during foraging flights. Until now, most research has focussed on honey bees, while data on pollutant loads of non-*Apis* pollinators is rare. *Bombus terrestris* is a common pollinator in cities and agrarian landscapes and has a more spatially restricted foraging behaviour. This makes *B. terrestris* well suited as a bioindicator representing the exposure of pollinators to various chemical profiles in anthropogenically influenced landscapes at high spatial resolution. In this study, commercial *B. terrestris* hives were placed at six locations in and around the city of Aachen, Germany, three in urban and three in agrarian surroundings. Foragers were caught when returning to the hive one and two month after placement of the hives. Individual bumblebees were extracted using a modified QuEChERS method and analysed for 26 target pesticides using HPLC-MS/MS. At every sampling location, at least one of the target pesticides was found. The results show that the individual pesticide load of bumblebees is variable between individuals from the same site (containing no pesticides up to five pesticides). This could be caused by individual foraging decisions and indicate complex pollution patterns in small scale habitat structure. No location or land-use specific pollution patterns were found over time. Rather, pesticide residues changed with the sampling time. Urban bumblebees had a significantly higher weight, possibly an indicator for a higher foraging success in the city compared to the agricultural fields. The ubiquitous presence of pesticides shown in this study should be taken into account when evaluating the risks to pollinators not only in Germany, but in all of Europe.

3.17.P-Tu186 Concentrations of Two Bisphenols, Three Parabens and Eight Perfluoroalkyl Compounds in Hair From 114 Volunteers From Liege, Wallonia

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Today, biomonitoring is principally performed using blood or urine. Blood is generally considered to reflect cumulative exposure especially for persistent organic pollutants. On the other hand, urine is commonly used for biomonitoring of rapidly eliminated substances. Beside these two main matrices, hair has gained attention as an alternative matrix for biomonitoring of environmental

pollutants because of the easiness of sample collection and storage, the extended detection window, and the possibility to reflect cumulative exposure. Even though this last decade the number of studies focused on hair biomonitoring of organic pollutants has increased, for certain chemicals hair concentration values remain scarce and many geographical regions haven't been covered yet. Therefore there is a need to provide data of hair biomonitoring from different regions and by different laboratories. This work presents the results of the first monitoring study of 2 bisphenols, 3 parabens and 8 perfluoroalkyl compounds in the hair from 114 volunteers (living in the city of Liege Belgium) using UPLC-MS/MS method. Each volunteer provided one hair sample and information related to age, gender, smoking status, hair treatments (none, dyeing, discoloration) and hair length (short, medium, long). Results showed high detection frequencies for six chemicals. The most frequently quantified compounds in the population were: bisphenol S (97.4%, median= 31.9pg.mg⁻¹), methylparaben (94.7%, median=28.9pg.mg⁻¹), bisphenol A (93.9%, median=46.6pg.mg⁻¹), ethylparaben (66.7%, median=5.2pg.mg⁻¹), propylparaben (54.8%, median=16.4pg.mg⁻¹) and PFOA (46.4%, median < 0.2pg.mg⁻¹). Other perfluoroalkyl compounds were rarely detected. Few statistical analyses were performed and only age was positively correlated with propylparaben concentration in the whole population and this correlation was strengthened in women group. Although blood seems to remain the most suitable matrix for exposure assessment to perfluoroalkyl compounds, our results demonstrate that hair analysis would be a promising approach for future biomonitoring studies of bisphenols and parabens.

3.17.P-Tu187 Is There Any Potential Role of Endocrine Disruptors on the Development of Gestational Diabetes Mellitus? A Pilot Study of INSULIN Cohort (Catalonia, Spain)

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The incidence of Gestational Diabetes Mellitus (GDM) has increased in the past 20 years, and it is now diagnosed in up to 7-10% of all pregnancies. This is a significant current public health concern, as GDM is associated with adverse consequences, including preeclampsia, birth complications, and cesarean delivery, as well as with long-term risk of type 2 diabetes mellitus, obesity, and cardiovascular disease for both the mother and child. A number of environmental factors can cause beta cell stress, including the developmental exposure to Endocrine-Disrupting Chemicals (EDCs). The role of EDCs on the development of GDM is a topic of recent concern, but with inconclusive results yet. The present pilot study was aimed at assessing the exposure to EDCs of pregnant women invited to join INSULIN, a cohort of gestational diabetes built at Hospital Joan XXIII (Tarragona, Catalonia, Spain). Sociodemographic, dietary and life-style determinants were linked to the occurrence of the EDCs and clinical data, including the development of GD during pregnancy. The occurrence of BPA and 10 BPA analogues (BPF, BPS, BPAF, BPAP, BPB, BPE, BPG, BPM, BPP, BPZ), phthalates metabolites (mono-ethyl, mono-butyl and mono-methyl); and parabens and their metabolites (methyl-, ethyl-, propyl- and butyl-) were assessed in urine (from the 12-week of pregnancy and delivery), mother blood (delivery), cord blood and/or placenta of 40 pregnant women. High detection rates of BPs, phthalate and parabens were found in urine and mother blood, but also their detection in placenta and cord blood, might be slightly related to the use of plastic and personal care products, along with the consumption of canned foodstuffs according to the results of the individual questionnaire filled on the 30-week of pregnancy. Albeit the premature state of the results do not allow identifying a potential role of a target EDC (or a mixture of EDCs) on the development of GDM, we can confirm the widespread presence of these EDCs in our daily life, which might become a hazard for the health of vulnerable population groups, such as pregnant women and their newborns.

3.17.P-Tu188 Breastfeeding Infants' Exposure to Endocrine Disruptors and Neurotoxic Substances: Earlyfood Study

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It is well known that breastfeeding is a gold standard for new-born nutrition with several benefits for both infants and women. However, some pollutants, including endocrine disruptors and neuro-toxics, were detected in breast milk. Biomonitoring breast milk can give information on current chemical exposition of infants and, in addition, on current and historical exposure of breastfeeding mothers. In present study, the levels of several pollutants, including persistent organic pollutants (DDT, DDE, HCB, PCBs and PBDEs), an organophosphate pesticide (chlorpyrifos), bisphenol A, tetrabromobisphenol A, per and polyfluoroalkyl substances (including PFOA, PFNA and PFOS), toxic elements (such as As, Cr, Hg, Pb, and methylmercury-MeHg) and essential elements (such as Na, K, Ca) were analysed. Results showed that maternal characteristic such as age and BMI were able to influence the levels of POPs (DDT, DDE and PCBs). Smoking, including passive smoking, mothers presented higher levels of As, Cr and Mn in breast milk. Higher levels of PCBs were detected in Spain born and primiparous mothers compared to non- and multiparous women. Breast milk from low-income mothers presented higher DDT and DDE levels than high-income mothers. Intake of PFOS and PFOA through breastmilk are higher than tolerable daily intakes (TDI) established by EFSA. For chlorpyrifos, despite no safe exposure levels have been established, this compound was detected in several samples. Although the clear health benefits of breastfeeding, exposure to the cocktail of hazardous substances and their interaction and mixing effects must be considered. This cocktail of toxic substances is not exclusive of breast milk, but also infant formula. The adverse effects of these chemical mixtures (that include endocrine disruptors and neurotoxic substances) on babies' health are unknown. Thus, increase the necessity of future research in the field.

3.17.P-Tu189 Temporal Trends in Occurrence and Risk of Pesticides in European Streams

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Besides climate change and habitat deterioration, pollution with chemicals is an important agent of global change. In this regard, freshwater ecosystems are of particular concern, because of the importance of water security and freshwater biodiversity for human prosperity. Agrochemicals, such as pesticides are increasingly sold and applied all over the world and their usage is expected to increase with climate change. Pesticides enter the aquatic ecosystems through diffuse runoff from adjacent agricultural fields. This may lead to a constant occurrence of pesticides in freshwater systems and associated risks to organisms. However, the seasonal trends of pesticide exposure has not been studied over a larger spatial scaling, including a wide range of pesticides and countries. In the present study, we investigated the seasonal occurrence and risk of pesticides in streams across Europe. We therefore analyzed data from governmental monitoring programs in European streams. Upstream from the sampling sites we delineated stream catchments to calculate land use parameters therein. As a risk measure we calculated Toxic Units (TU) for different organism groups (i.e. algae, invertebrates, fish) as the decadic logarithm of the measured in stream concentrations divided by the median effective concentrations (EC50). We identified spring as the period with the most significant increases, exhibiting country-specific trends though. Additionally, increased occurrence was detected during end of summer and autumn months. Individual pesticides of the same class showed mostly similar trends in occurrence, though this did not hold true for all pesticides. Seasonal risk trends also showed significant increases during spring months. Lastly, we found that agriculture in the catchment strongly influences the occurrence and risk of pesticides in streams. Current monitoring data allows to delineate the seasonal presence of well tested pesticides as well as their associated risk trends. Though the latter might underestimate the actual risk. Modelling seasonal trends largely depends on the availability and quality of pesticide monitoring data, which currently differs a lot between European countries.

3.17.P-Tu190 Assessing the Presence of Pesticides in UK Bird Species

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The introduction of pesticides has dramatically altered the farming landscape, but they also contaminate and exert different effects on the surrounding environment. Few studies investigate the occurrence of pesticides in terrestrial birds. This study aims to identify and quantify the levels of pesticides from feathers obtained from birds from across the UK. Samples were collected by trained volunteers from 15 common bird species found in the UK. This study aims to contribute to previous studies that revealed the presence of neonicotinoids in house sparrow feathers. We aim to assess feather samples for the presence of 18 pesticides which include imidacloprid, prothioconazole and pendimethalin. Pesticide presence will be quantified using UHPLC/MS-MS. This study will highlight the levels of pesticide exposure in the selected bird species and the levels of contamination within the agricultural habitat.

3.18 Taking up the Nanoplastics Challenge: Materials, Methods and Impacts

3.18.T-01 Is Mechanical Degradation of Macroplastics a Suitable Approach for Obtaining Environmentally Relevant Nanoplastics?

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The occurrence of nanoplastics (NPs) in the environment is a matter of increasing concern and deserves proper attention. However, there is still a lack of analytical tools for straightforward monitoring of NPs at environmentally relevant levels in different matrices. In addition, environmentally relevant NPs are needed for further *in vitro* and toxicological studies, in which critical properties of the NPs present in the environment are very important. Consequently, the main aim of this study was to investigate whether high-energy ball milling of commercially available polyethylene perler beads containing Ti (6.1 g/kg) as a white pigment can be a potential route to produce environmentally relevant NPs. STEM images, DLS size measurements of mechanically produced polyethylene nanoparticle samples dispersed in 0.1% Novachem and transient signals and integrated intensity distributions obtained for the same particles measured using ICP-MS operated in single-event mode via the monitoring of ⁴⁷Ti⁺ suggested that mechanical degradation of macroplastics is indeed a possible route for the production of environmentally relevant NPs. However, our research also shows that during high-energy ball milling of macroplastics into environmentally relevant NPs, significant zirconium contamination occurs. As such, the NPs produced by using high-energy ball milling could not be used for *in vitro* and toxicological studies. Therefore, additional production routes are needed for providing NPs relevant for *in vitro* and toxicological studies.

3.18.T-02 Development of Test Methods and Standards for Separating and Characterizing Environmental NanoPlastics in Aqueous Media

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The impact of marine debris and plastic waste, including the formation of microplastics and nanoplastics (MNP), on environmental and human health are areas of growing concern. The exposure risk of these materials resulting from accumulation, degradation, and other changes to interfacial properties after release cannot be evaluated with current separation and quantification methods, especially in the sub-micrometer regimes. To begin addressing the measurement requirements necessary for more accurate risk assessment, parallel development of control test materials and the analytical tools, methods and guides, are necessary

to more accurately identify, characterize, and quantify MNP in complex matrices. Importantly, the control materials need to behave similarly to field collected samples (e.g., composition, colloidal properties, and size) to ensure method efficiency for targeted applications. This presentation will focus on the development of sample handling methods for cryomilled samples, produced from both controls and environmentally sourced plastics, generated through collaborative efforts to produce size-binned MNP samples in concentrations that are suitable for investigation with current spectroscopic and spectrometric methods. We will present the relative separation efficiencies obtained from fractionation and filtration methods for select, pure polymer controls, which will be used to compare the size distributions determined using light scattering and particle population analysis. Using isolated MNP fractions with characterized size distributions, we will present the size dependent properties and relative performance metrics for commonly implemented spectroscopy and spectrometric methods to improve reproducibility and examine heterogeneity for different preparation procedures.

3.18.T-03 Characterization of Nanoplastics, Fibrils, and Microplastics Released During Washing, Abrasion and Weathering of Polyester Textiles

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Nanoplastics (defined here as plastic particles smaller than 1000 nm) released during the daily use of plastic products are gaining increasing attention due to their potential effects on human and environmental health. Formation of nanoplastics has been reported so far for diverse plastic products under varying conditions of use. The washing of synthetic textiles has been identified as an important source of microplastic fibers (MPF) released to the environment. In addition, abrasion of textiles was shown to induce further fragmentation of fibers and subsequent formation of much smaller and shorter fibrils. The formation of micro- and nanoplastics during weathering was rarely investigated. The aim of this work was to identify whether washing and wearing of textiles also results in the formation of nanoplastics. We designed washing, abrasion and weathering experiments to investigate the morphology, number, and size of micro- and nanoplastics released from polyester textiles. Using a combination of techniques including scanning transmission X-ray microspectroscopy (STXM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and nanoparticle tracking analysis (NTA), we were able to quantify nanoplastics (average hydrodynamic diameter 173–188 nm), microplastic fibrils (diameter $3 \pm 1 \mu\text{m}$, length 20–160 μm), and MPFs (diameter $16 \pm 7 \mu\text{m}$, length up to 5 mm). The presence of polyester nanoplastics was confirmed by the near edge X-ray absorption fine spectra (NEXAFS) of the nanoparticles in the abrasion and washing samples for particles larger than 100 nm. We estimated that in the abraded samples, 1 g of fleece textile released an average of 2.1×10^{11} nanoplastic particles (1.4 mg), 1.4×10^4 MPFs (1.0 mg), and 5.3×10^5 fibrils (0.5 mg) based on SEM images and NTA. In the nonabraded samples, 1 g of textile released an average of 3.3×10^{11} nanoplastic particles (2.1 mg), 2.8×10^3 MPFs (0.2 mg), and no fibrils. The present study is the first to show a significant release of polyester nanoplastics during the washing and abrasion of synthetic textiles.

3.18.T-04 Quantification of Nanoplastic Uptake Into the Across the Fish Intestine Using Palladium-Doped Nanopolystyrene Particles

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Fish are widely reported to ingest microplastics with low levels accumulating in the tissues, but due to analytical constraints, much less is known about the accumulation of nanoplastics via the gut. Recently, the labeling of plastics with inorganic metals (e.g., palladium) has allowed measurements of nanoplastic uptake. The aim of the current study was to quantitatively assess the uptake of palladium-doped nano-polystyrene particles (PS-Pd NPs) by the fish gut. The first experiment used an *ex vivo* gut sac system, whereby the entire gastrointestinal tract of fish was removed, and the anterior, mid and hind intestine regions were separated into each compartment. Each compartment was filled with either an unexposed control (gut physiological saline) or saline containing PS-Pd NPs ($1 \mu\text{g Pd mL}^{-1}$). The gut sacs were exposed for 4 hours and then dissected into the mucosa, muscularis and serosal saline for Pd measurements via inductively coupled plasma mass spectrometry (ICP-MS). The second experiment was an *in vivo* feeding study, whereby fish were fed either an unexposed control (no added Pd), or 10 mg Pd kg^{-1} as PS-Pd NPs for 7 days and then sampled for internal tissues. Using single particle ICP-MS, the particle number concentration for a given Pd mass concentration was calculated and used to determine the number of PS-Pd NPs in both experiments. The gut sac showed that in 4 hours between 200-700 million nanoplastics (2.5-9.4% of the administered nanoplastics) can enter the mucosa and muscularis layers of the intestine. Of the particles taken up, up to 700,000 of the nanoplastics (0.6%) cross the gut epithelium of the anterior intestine and exit into the serosal saline. These data provide a proof-of-concept study, suggesting the potential for nanoplastics to distribute throughout the body. The uptake of the PS-Pd NPs was confirmed in the *in vivo* experiment. After 7 days, there was detectable Pd in the PS-Pd NP treatment. For instance, the kidney and carcass had total Pd concentrations of 65.6 ± 25.4 and $2.2 \pm 0.4 \text{ ng Pd g}^{-1} \text{ dw}$. When the Pd mass concentrations were converted to number of particles, the total number of particles accumulated after 7 days into the fish was $34.2 \pm 6.6 \times 10^6$ particles. In summary, our studies are the first quantitative assessments of the uptake of nanoplastics by the fish gut, and the presence of nanoplastics in the carcass raises concerns around the potential trophic transfer of nanoplastics to humans.

3.18.T-05 Assessment of Drinking Water Treatment Processes in Nanoplastics Removal: Laboratory-scale, Pilot- Scale and Modelling Studies

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Microplastics have been detected in both potable water sources and tap water, leading to questions about the efficacy of current water treatment practices to remove these particles. Due to analytical difficulties, the detection of nanoplastics (NPs) both in the environment and in potable water is still challenging, however, it is hypothesized that water resources already contain NPs. The aim of this study was to investigate the NPs removal efficiency through several stages of the drinking water treatment plant (DWTP). Using metal doped NPs allowed us to quantify these particles in the treated water and in the filter media, where they were possibly retained. We measured breakthrough curves of NPs through sand and activated carbon filled columns in the laboratory and pilot-scale equivalents under different conditions to understand the processes which impact NPs retention. The MNMs software (Micro- and Nano-particles transport, filtration and clogging Model Suite) was used to model the breakthrough curves obtained from the pilot-scale DWTP to derive the hydrodynamic parameters of the filtration systems and used to simulate the behaviour of NPs in full-scale DWTP. In laboratory-scale column experiments, the filter length and flow rate impacted NPs retention in the filter media (sand and activated carbon). We assessed both pristine and aged sand and activated carbon, where the presence of a biofilm, which naturally occurs on the filter media, further reduced the concentration of NPs in treated water. In agreement with laboratory studies, results from the pilot-scale DWTP showed higher retention of NPs in the sand filtration opposed to activated carbon, in part due to the increased biofilm layer (Schmutzdecke). Both experimental and modeling results indicate a high capability of the filtration units in DWTPs to remove NPs from the source water across the entire treatment chain (>99%), and the slow sand filtration dominated NPs removal (3.6 log removal). As slow sand filtration systems are slowly decreasing in popularity due to space and economic requirements for upkeep, this highlights the importance of testing other drinking water treatment configurations for their efficacy of removing NPs throughout the water treatment chain. The results of this study can serve as a baseline for assessing the performance of DWTPs to remove NPs from polluted water sources and the capability of current water treatment infrastructure to provide plastic-free, potable water.

3.18 Taking up the Nanoplastics Challenge: Materials, Methods and Impacts (Virtual Only)

3.18.V-01 Three Stage Size-Selective Nano- and Micro-Plastic Particle Porous Membrane Filter System for Analytical Workflows

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A quantitative metrology and testing of nano and micro-plastic (NMP) particles in liquids and air requires suitable representative methods for sampling, preparation and detection for all NMP size ranges from 50 nm to larger than 150 µm. For this purpose, filter materials have to be configured to allow a size selective analysis of NMP particles and polymer types and implemented in the analytical workflows. Analytical routes for particle sizes in the range of several 10 µm are nowadays well established, such that current scientific efforts are focused on entering nano particle dimensions with diameters down to 10 nm. A major challenge lies in the availability of cascade filter setups spanning several orders of magnitude in a single filtration workflow with appropriate NMP analytic routines. In this work, we are presenting a three-stage cascade filtration system, that should be used for water as well as for air filtration. The corresponding analytical workflow will be implemented and tested on well-defined NMP test material. The NMP test material is composed of a mixture of PET micro plastic reference material, monodisperse PMMA particles and monodisperse PS particles. The novel cascade filter system contains three filters made of microporous and nanoporous Si as well as nanoporous aluminum oxide (AlOx) membranes, thus realizing the required NMP size selectivity. In our experiments we demonstrate the successful NMP size separation on Si and AlOx filter substrates. Using the filters subsequently as analytical substrates, leads to an enhanced flexibility in the application of µRaman, SEM, and AFM instrumentation. We are able to analyze NMP properties, e.g. particle shape, size, spectroscopic fingerprints - optical and chemical, both qualitatively and quantitatively. Finally, requirements and limitations for a standardized NMP filtration and analytic workflow will be discussed.

3.18.V-02 Understanding NanoPlastics Interaction With Minerals of Freshwater Weathering Sequence in Riverine Environment

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Recent scientific evidence predicts the massive accumulation of nanoplastics (NPs) in freshwater, especially in a riverine environment. The majority of NPs research has highly focused on the marine environment, knowing the fact that rivers are major carrier of NPs to the oceans, very handful of work has been done in understanding NPs behavior in a riverine system. River water continuously erodes the rock's surface and releases suspended minerals fraction in water, which can interact with NPs and further impact their bioavailability and transport to the oceans. Keeping this natural scenario in mind, for the first time, we sought to investigate NPs interaction with a typical weathered sequence of minerals in the river water (fig. 1a) under varying environmental conditions. Most abundant, primary mineral feldspar and its weathering originated secondary minerals, i.e., kaolinite and gibbsite, were investigated for interaction with NPs using aggregation and batch sorption experiments. The impact of environmental parameters like varying major ions, pH, humic acid, and natural aqueous matrix, i.e., river water, on the mineral-NPs interaction was studied in detail. Experimental data modeling using non-linear kinetic and isotherm models and theoretical calculation using DLVO theory were further performed to enhance understanding of minerals-NPs interaction. Adsorption isotherm results (fig. 1b) revealed that, gibbsite exhibits maximum sorption of NPs due to its amorphous nature, large size, and positive surface charge. Whereas, continuous adsorption-desorption and limited sorption capacity of feldspar and kaolinite can be attributed to their

negative surface charge, larger size, crystalline nature, and physical sorption. Also, microscopic analysis studies suggested strong gibbsite-NPs interaction. This work also revealed that apart from surface charge of minerals, their size, crystalline and amorphous nature also play a vital role in adsorption of NPs. Overall, this study provides a clear insights of NPs interaction with abundantly occurring minerals (feldspar, kaolinite, and gibbsite) in riverine environments, which can ultimately govern the fate and transport of NPs.

3.18 Taking up the Nanoplastics Challenge: Materials, Methods and Impacts (Poster)

3.18.P-Tu191 Interaction of Marine Algae and Nanoplastics, and the Possible Impact on the Bioavailability of Nanoplastics to Primary Consumers Through the Food-Pathway

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Plastic pollution is a global problem. While macro- and microplastics are largely studied in terms of environmental concentrations and toxicity, a knowledge gap exists regarding nanoplastics (1-1000 nm diameter). These enter our environment both as primary products, engineered for cosmetic and pharmaceutical purposes, and as secondary degradation products. However, due to deficient sampling methods and analytical identification methods, information on environmental concentrations is lacking. Nevertheless, it is assumed that the exposure route, extent and rate of bio-uptake, and the nature of adverse effects will differ from those of microplastics, due to the reactivity features of nanoscale entities and the fact that they are small enough to cross biological barriers. The aim of the study is to investigate the interaction between marine phytoplankton species and nanoplastics. We want to analyze whether there is an interaction, and what sort of interaction (aggregate formation, adsorption of the particles on the algal cell surface or absorption of the particles into the algae cell). Subsequently, we will analyze EPS production to investigate whether algae-aggregate formation is affected by the nanoplastics' presence. Analysis of the quantity of particles that interact with algae is done using flow cytometry and visual inspection of interactions was performed using fluorescence microscopy. The algae species used for the experiment were *Rhodomonas salina* and *Isychrysis galbana*, which are relevant algal species for the North Sea food web. Both species are exposed to concentrations of nanoplastics, that were assumed to be environmentally realistic, using data on micro-plastic concentrations in the North-Sea and the conversion factor of 10^{14} as proposed by Besseling et al., 2019. This factor is based on mass conservation principles, for the fragmentation of spherical particles with a size of > 0.1 mm – 5 mm into 100 nm particles. The nanoplastics used are fragmented hydrophilic PET and polystyrene with a broad unimodal size distribution: diameter in the range from 40 nm to 5 μ m. As a control, non-plastic nanoparticles will be used to differentiate between plastic-specific and particle-specific effects. This research will be valuable for studying the effect of algae-NP interaction and provides relevant information on algae as a potential exposure pathway for marine copepod species such as *Nitocra spinipes* and *Acartia tonsa*.

3.18.P-Tu192 Generation of Test Nanoplastics From Consumer Products Through Cryomilling

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Concerns over increasing levels of environmental plastic pollution have led to advances in the production and use of biologically-based plastics, with the biodegradable fraction of these materials showing promise in alleviating environmental risk. The evaluation of risk requires toxicity evaluation; however, at the nano-scale, very few test materials are available and most commercially available nanoplastics are spherical polystyrene. We investigated methods for generating nano-scale test materials through cryomilling and fractionation of consumer products including traditional petroleum-based polypropylene (PP) drinking straws and bio-based polylactic acid (PLA) drinking straws. Test materials were first generated as dry cryomilled materials, then suspended in various waters and fractionated using 1 μ m filters to generate nano-scale test materials (< 1000 nm), and then quantified using nanoparticle tracking analysis (NTA). In addition, materials were generated in both ceramic and stainless-steel milling chambers to test for potential metal contamination by the stainless chambers. Lastly, we investigated methods for concentrating the nano-scale particles using dialysis in a polyethylene glycol (PEG) solution. The results showed the both PP and PLA straws did generate nano-scale particles following cryomilling with the PLA making significantly more nanoparticles per unit initial mass than PP (2.9×10^9 and 4.7×10^8 particles/mL, respectively). Particle sizes as measured by NTA were not significantly different with diameters of 201 and 180 nm for PP and PLA, respectively. Extraction of the materials with concentrated nitric acid and subsequent ICP-MS determinations showed no significant metal contamination was found from the use of the stainless-steel chamber relative to ceramic. The estimated mass-based concentration of nanoparticles generated is 0.2 μ g/ml for PP and 1.1 μ g/ml for PLA from 1 g cryomilled material per 45ml suspension solution. Although these concentrations are low, the nano-scale particles can be further concentrated using dialysis with PEG to remove the water and concentrate the particles. Thus, the generation of nano-scale plastic test materials can be accomplished; however, techniques need be further optimized to generate higher concentrations of nano-scale test particles to facilitate concentration-response testing.

3.18.P-Tu193 Advancing Our Understanding of Plastic Fragmentation in the Environment Through a Mechanistic Model of Micro- and Nanoplastic Fragmentation

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The potential for plastics to degrade and fragment towards nano-sized particles in the environment is raising concerns about the divergent fate, hazard and ultimately risk of different sized plastic particles. For example, nanoplastics may be more readily able to permeate biological membranes, and they may follow different fate pathways enabled by their potential to aggregate to natural environmental particles. However, there is currently limited understanding of the fragmentation process, including the influence of different environmental stresses in altering the susceptibility of plastics to fragment. We are addressing this by developing an open-source mechanistic model of Micro and NanoPlastic degradation and FRAGMENTation in the ENvironment (FRAGMENT-MNP). Our model is underpinned by an experimental database of how different environmental factors - such as UV light, hydrolysis, temperature and biodegradation - alter the susceptibility of a broad range of polymers to fragmentation by mechanical shear, and is validated by assessing the long-term fate of polymers exposed to combinations of these stresses in realistic environmental media. FRAGMENT-MNP aims to be pragmatic in terms of the information users must provide, thus making it a useful tool for stakeholders, such as industry and regulators. To further enable this, guidance on protocols to obtain the required input information and using the model is being developed. This pragmatism and a focus on technical interoperability will enable its integration into other models, such as those for fate and exposure. Herein, we will present the first version of FRAGMENT-MNP and its interface, including indicative results demonstrating the use of the model and highlighting the importance of certain parameters in determining plastic fragmentation. Ultimately, this work will help us gain mechanistic insights into fragmentation rates and resulting size distributions of various polymers under realistic environmental conditions, providing a significant step forward in our understanding of plastic fragmentation and nanoplastic pollution in the environment.

3.18.P-Tu194 NanoRelease: Microplastic Fragmentation Rates, Degradation and Dissociation for Modeling Purposes

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Nanoplastics are suspected to behave differently from microplastics, leading to increasing concerns due to their potentially higher bioavailability. Insights into fragmentation and degradation mechanisms of microplastics into micro- and nanoplastic fragments and other degradation products are still limited and literature barely provides data on fragmentation and degradation rates. For this reason we adapted the NanoRelease protocol (originally developed to quantify released fragments from macroplastics under weathering) to simulate UV aging of microplastics at the beach and mechanical abrasion in the surf zone for a UV-dose-dependent assessment and size-selective quantification of micro- and nanoplastic fragments released after environmental degradation. We determine the amount, shape and sizes of released polyamide-6 (PA-6) and thermoplastic polyurethane (TPU) fragments and dissolved organics with increasing duration of UV irradiation, as well as the chemical properties of the polymers themselves. Our test materials are relevant for primary microplastic powders commercialized for additive manufacturing. But since microplastic fragmentation and degradation strongly depends on the chemical composition, the established protocol can be applied for all types of polymers. The used methods include analytical ultracentrifugation, UV-Vis spectroscopy, scanning electron microscopy, Fourier-transformed infrared spectroscopy, total organic carbon and gel permeation chromatography. The measured rates can serve to validate mechanistic microplastic fragmentation models (FRAGMENT-MNP, Cefic LRI) and can be used to parameterize microplastic fate models that include degradation terms (The Full Multi). Pragmatic approaches (e.g. assuming a shrinking sphere by layered degradation of a specific thickness in a specified time) combined with the generated data can serve for micro- and nanoplastic half-time predictions. This can be relevant when selecting longer lasting materials for certain applications to evaluate and to improve their sustainability.

3.18.P-Tu195 Detection and Formation Mechanisms of Secondary Nanoplastic Released From Drinking Water Bottles

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Since nanoplastics are currently considered potentially hazardous to the environment and human health, reliability of studies on nanoplastic exposure becomes crucial. However, analytical challenges limit our understanding of their formation and detection, thus hampering their biological interactions assessment. Here we provide a combined approach to quantitatively and qualitatively detect the release of nanoplastics in water matrix and, in particular, to measure direct exposure of consumers by simulated use of drinking water plastic bottles. We measured that the polyethylene sealing of the bottles released particles with a size distribution ranging from few hundreds nanometers up to about one micron and estimated a mass release in the order of few tenths of nanograms per opening/closing cycle. We observe that mechanical stress alters the physical-chemical characteristics of the generated secondary nanoplastics and degrades the material properties compared to the original bulk source, thus complicating their spectroscopic chemical identification. Our findings demonstrate that understanding material degradation processes is therefore crucial for identifying and quantifying nanoplastics in real samples. Moreover, methods allowing quantitative studies on the release of nanoplastic as a source of exposure are considered essential for proper assessment of their potential health hazards and to promote improvements in consumer products plastic packaging design.

3.18.P-Tu196 Methodological Development for the Characterization of Small Microplastics and NanoPlastics in Seawater

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The release of plastics into the environment is universally recognised as a major threat not only to terrestrial and marine ecosystems, but potentially also to human health. Their increasing presence in the environment is causing global concern about potential risks to human health through contamination of food, water, soil and air [1]. This concern is mainly related to the degradation of plastic materials into micrometer and nanometer sized particles that can more easily accumulate in the body. Plastic particles are present in all environmental compartments, particularly in soils, sediments and waters, deep and superficial, marine and continental. Quantitative data on these particles are still scarce and not very conclusive because the studies are recent and the measurement protocols are not harmonised. Despite the large number of papers in the field, the research topic is very young with little stabilised methodologies and standardised analytical approaches. The use of different analytical methods and units of measurement in scientific reports and studies for understanding the impact of pollution on ecosystems and human health makes it difficult to compare results and exploit existing data. The MICROPLAST project, financed by the ACTIA 2020 programme, in connection with RMT ProPack Food, a French network for food technology institutes, was focuses on the evaluation of sampling and characterisation methods for micro-nano-plastics disseminated in the marine environment, supporting the sampling campaigns in the Mediterranean Sea carried out by SEA Plastics. SEA Plastics is an organization led by engineering students, to contribute to research on microplastics and to raise awareness about plastic pollution. The aim of the work was to define an analytical strategy to extract/purify/concentrate MP from seawater samples, separate them according to their size using Asymmetrical Flow-Field Flow Fractionation multidetector system (AF4-MD) and characterise the size, shape, chemical identity and concentration by Raman microscopy and pyrolysis coupled to gas chromatography mass spectrometry (pyr-GC-MS).

3.18.P-Tu197 Towards the Analysis of Real Samples Using Online-Coupled Field-Flow Fractionation-Raman Microspectroscopy: Importance of Suitable Reference Materials

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While several techniques have been developed in the past years to face the challenge of nanoplastics analysis, proper development, optimization and validation require suitable reference materials. Commercially available materials are often limited to monodisperse, spherical particles and a small material range. These reference particles are not expected to resemble particles found in real samples. Shape, particle size distribution, material properties, ageing state and surface chemistry is presumed to vary a lot in environmental samples. These differences between real samples and references can affect sample preparation and detection methods alike. To emphasize this issue, reference materials which can better emulate environmentally relevant nanoplastics than the previously used monodisperse, spherical (plastic) particles were tested in a recently developed online-coupling of field-flow fractionation (FFF) and Raman microspectroscopy (RM). Online FFF-RM system allows for the analysis of (plastic) particles in a size range from 100 nm to 5 μ m, which is not fully accessible to common optical microscopy methods. Specifically, the FFF system is used to separate particles (where multi-angle light scattering (MALS) detector can provide the corresponding size information) while chemical identification is performed by RM. A flow cell enables the chemical characterization via RM in an online-coupled mode to the FFF systems. This online detection is enabled by the optical forces of the Raman laser (Raman tweezers) where particles are trapped in the focus of the laser beam and held long enough for acquisition of spectra. In this work online-coupling of centrifugal FFF (CF3, which allows for the separation of particles according to their size and density) and RM is successfully applied for the size-resolved chemical identification of reference particles (including mono- and polydisperse nanoplastics made of PE, PS and PMMA as well as various inorganic nanoparticles) in different mixtures. RM-based identification of rod-shaped particles using this technique remains challenging, however. This might be explained by the requirement of stronger optical trapping for these shapes. It underlines (i) the need for further setup development (ii) involving suitable reference materials. The information gained here can support this development: to detect, and choose more realistic reference materials to foster the proper development and optimization of (novel) analytical techniques.

3.18.P-Tu198 Analysis of Small Microplastics and NanoPlastics in Complex Matrices: Towards a Strategic Toolbox

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Plastic pollution is ubiquitous and persistent in the aquatic environment where a mixture of plastic particles is found. Environmental plastic particles are heterogeneous in terms of both physical (e.g. size, shape, density, porosity, colour) and chemical properties (e.g. polymer type, surface charge, polarity, presence of additives or persistent organic pollutants). A strong international research effort has been made over the past decade, notably on methods development, to analyse plastic items with dimensions between 1 μ m and 5 mm known as microplastics (MPs). However, MPs will eventually degrade in the environment - with a concomitant modification of their initial characteristics - to successively smaller entities, eventually reaching the nano-size range: they are then denoted as nanoplastics (dimensions < 1 μ m; NPs). Nanoplastics are expected to be widely present in the environment, but there is a paucity of reliable data on the concentration and distribution of small MPs (dimensions < 10 μ m) and NPs in environmental matrices. These small particles are less investigated notably because of sampling and analytical challenges. Concomitantly, there are knowledge gaps in understanding their effects and interactions with biota. Indeed, based on their size, NPs are able to cross biological barriers and thus have the potential to bioaccumulate and induce long-term effects on biota. Bioaccumulation of NPs also poses a potential risk for human health if accumulation occurs in tissues that are consumed by

humans. In such case there may also be economic consequences when economically important marine and estuarine species are involved. The current lack of relevant and standardized method(s) for quantification of MPs and NPs precludes the development of robust environmental risk assessment strategies. The objective of the present work is to first identify a suite of reliable and efficient methods for determination of the concentration and distribution of small MPs and NPs in complex environmental matrices including information on their physical and chemical characteristics. The developed analytical toolbox will provide the means to establish realistic input values for further ecotoxicological studies and risk assessment regarding plastic particles with size smaller than 10 µm.

3.18.P-Tu199 Disentangling Fate and Uptake of Plastic Nanoparticles in Semi-Natural Wetland Ecosystems

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Given the tremendous amount of plastic waste, there is a need to assess the fate and environmental effects of nano-plastic particles (NPPs) in natural ecosystems. We have used metal cored NPPs to track and quantify them using standard methods for trace metal analytics (e.g., inductively coupled plasma mass spectrometry (ICP-MS)). In this study, we aimed to evaluate the transport, fate and uptake of NPPs using constructed freshwater wetlands and polystyrene nanoparticles labelled with a gold core (size: 88 ± 11 nm). Twelve constructed wetlands were set up in glass aquaria in a greenhouse. The wetlands were divided in two areas: a small lake where *Asellus aquaticus* and *Daphnia magna* were introduced, and a sediment area with macrophytes originating from natural wetlands. Each wetland was fed with tap water (3 ml/min) using a peristaltic pump, that flowed through the wetland towards an outlet in the opposite side. The NPPs used had an Au core (size: 13 ± 1 nm) surrounded by SiO₂ and a polystyrene shell with a negatively charged surface. NPPs were added weekly for 10 weeks to the lake area of the treatment wetlands (n = 6, randomly assigned; NPPs concentrations: approx. 0.2 mg/l of polystyrene). Water samples from each wetland's lake were collected weekly, and from the outlet were taken three times per week throughout the experiment. Furthermore, at the end of the experiment (week 10), animals, sediment and macrophytes were sampled. All samples were analysed using ICP-MS to quantify NPPs distribution within the system. We show that although some NPPs moved through the wetland following the NPPs pulses, a considerable part of the NPPs were retained in sediment and in macrophytes roots, and interestingly, significantly more than in macrophytes leaves. In addition, NPPs were taken up by both *D. magna* and *A. aquaticus*, but *D. magna* showed a significantly higher uptake. Overall, the present study can contribute to determine which environmental process might guide the fate of NPPs in natural freshwater ecosystems.

3.18.P-Tu200 Micro- and Nanoplastic Transfer in Freezing Saltwater: Implications for Their Fate in Polar Waters

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Plastic debris accumulates in the Arctic Ocean by way of oceanic and atmospheric circulation. High concentrations of microplastics (1 µm to 5 mm) have been measured, and nanoplastics (< 1 µm) are expected to be abundant as well. Microplastics accumulate in sea ice compared to the underlying seawater. However, due to the technical difficulty of quantifying nanoplastics in environmental matrices, the fate of nanoplastics at this seawater/ice interface is unknown. Therefore, experimental approaches that model this environmental interface and use models of nanoplastic particles are essential to fill this knowledge gap. This study investigates the fate of micro- and nanoplastics during sea-ice formation. A novel experimental setup simulates the growth of sea ice by progressively freezing a saline solution. After different durations of freezing, the concentrations of NaCl, natural organic matter, microplastics, and nanoplastics were measured in the ice and liquid. Micro- and nanoplastic distribution coefficients between saltwater and ice were determined, reflecting their behavior during congelation sea-ice growth. The results show that microplastics are retained in ice while nanoplastics are expelled from it. This is due to the colloidal properties of nanoplastics, which confer them a high diffusion coefficient. Furthermore, natural organic matter plays a crucial role in stabilizing nanoplastics at this interface. This highlights the fact that due to their different properties, the environmental fate of submicrometric plastic debris must be studied separately from that of microplastics. These results raise new questions concerning the impact of micro- and nanoplastics in fragile polar environments and the analytical strategy to detect them. This study has been published in Environmental Sciences Processes and Impacts <https://doi.org/10.1039/D1EM00280E>

3.18.P-Tu201 Ecotoxicity Assessment of NanoPlastics on Environmentally Relevant Planktonic Species: Does the Biological Filter Mesh-Size Influence Bioconcentration of Nanometric Plastic Particles?

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Size-assorted plastic debris incidence is an environmental threat currently acknowledged worldwide, even in the most remote biomes [1]. Together to their persistency [2], their high specific surface area makes nanometric plastic particles (nanoplastics) more likely to pose an ecotoxicity risk owing to the impact of their greater reactivity (given their inferior size) [3] on their environmental fate [4] [5]. Taking into account that any particulates below 100 nanometers are more prone to cross relevant biological barriers, “nano” ought to be the size range to target when undertaking an ecotoxicity assessment. However, unequivocal cellular and systemic (eco)toxicity evidence associated to nanoplastics exposure have yet to be demonstrated. Most studies reporting ecotoxic data do not test environmentally relevant nanoplastics nor do use environmentally relevant (nominal)

concentrations. In this work, environmentally (aquatic) relevant planktonic species were exposed to different (standard, synthesized and cryomilled) nanoplastics (size range < 1 µm) for 24 h, considering nonstandard experimental designs (Figure 1). Dynamic light scattering and zeta potential measurements had been previously performed to study aggregation/agglomeration of nanoplastics on ultra-pure water and on microorganisms' specific culture media. Data on microorganisms/nanoplastics interactions were gathered by dark field microscopy, SEM and TEM analysis. Taken together, these results pinpoint the need to refine and reorient the perspective on where and what to look at when assessing nanoplastics aquatic toxicity.

3.19 Trace element biogeochemistry, exposure and impacts in ecosystems (Part I)

3.19.T-01 Copper Sources to Water: An Apportionment Exercise for the EU27

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Although large emitters report for priority chemicals through the European Pollution Release and Transfer Register (E-PRTR) little is known on a Member State scale regarding total emissions to water including diffuse as well as point sources. This project focussed on estimating loads to water for copper for all significant sources including industry, sewage treatment plants, surface runoff (from traffic, architecture and atmospheric deposition), septic tanks, agriculture (from plant protection products, biosolids, fertilisers, manures and atmospheric deposition), aquaculture, boat transport (from antifoulant leaching), natural processes (atmospheric deposition, groundwater sources and soil loss). To achieve this, a combination of European datasets (Eurostat databases), literature and industry data were utilised to generate export coefficients which could be multiplied by activity rates to generate loads. A total of around 8,000 tons of copper per annum was estimated to enter freshwaters of the EU27, with around half of that (3,849 tons per year) entering transitional and coastal waters. The main inputs to freshwater were natural processes, predominantly copper associated with soil loss (2,900 tons per year) followed by agricultural and urban runoff at 1,848 and 1,839 tons per year, respectively. Agricultural emissions were dominated by the use of copper in biocides used in vineyards, particularly high for Italy and Spain, and farmyard manure. Urban runoff was dominated by copper use in brake linings followed by architecture, which is prevalent in Germany, Italy and Austria. Antifoulants from boats (3,561 tons per year) dominated saline water loads of copper. Calculated as a per capita basis, loadings to water ranged from 10 (Belgium) to 68 (Latvia) grams Cu per capita per year, the latter's high value reflected high rates of shipping associated with its Baltic ports. Where possible estimates of uncertainty were performed, highlighting a lack of confidence in data from abandoned mines and many variables and assumptions relating to urban runoff load estimates. For the first time, this study has quantified sources across an exhaustive list of point and diffuse inputs of copper to the aquatic environment, which have been combined to generate loads on a per country basis, summed to provide continental scale estimates.

3.19.T-02 Dynamic and Equilibrium Speciation Information Can Be Integrated to Predict Availability

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Chemical speciation has been, since long ago, recognized as a key predictor of biouptake and toxicity of many elements. The Free Ion Activity Model (FIAM) and the Biotic Ligand Model (BLM) highlight the relevance of the free form of the metal ion (i.e. just the hexa-aquo complex) and correspond to the equilibrium speciation approach. Several analytical techniques to provide free metal ion concentrations have been developed, such as Ion Selective Electrodes (ISE), Ion Exchange Technique (IET), Donnan Membrane Technique (DMT), etc. Alternatively, dynamic speciation, focussing on the flux of toxicant/nutrient reaching the biota/sensor, has been suggested to be more relevant in some systems. Among the analytical techniques for dynamic speciation, we find Scanned Stripping Chronopotentiometry (SSCP), Dynamic Ion Exchange Technique (DIET), Permeation Liquid Membrane (PLM), etc. For each dynamic technique, its diffusion length plays a crucial role in determining its resulting flux and the ensuing lability degree. Comparison of results from different techniques has been hindered by the fact that equilibrium techniques provide a concentration, while –in general- dynamic techniques provide a flux. In this contribution, we are going to define a labile concentration for each dynamic technique and integrate equilibrium and dynamic concentrations in a single plot, called “effective concentration signature”. This plot, combined with the characteristic lengths of the techniques, will provide estimates for the maximum available supply of the element that a medium can provide to an organism from a known (or estimated) characteristic diffusion length for the considered uptake. These new concepts will be illustrated for the particular case of Zn in a Mediterranean stream (Osor), using 5 analytical techniques: AGNES (Absence of Gradients and Nernstian Equilibrium Stripping), PIM (Polymer Inclusion Membrane), LASV (Linear Anodic Stripping Voltammetry), DGT (Diffusion Gradients in Thin-films) and inductively coupled plasma mass spectrometry (ICP-MS). *Acknowledgement* - Support from the Spanish Ministry of Science and Innovation (Project PID2019-107033GB funded by MCIN/AEI/10.13039/501100011033), from UdL (JSD) and from FISDUR-Generalitat de Catalunya (KRS).

3.19.T-03 Prediction of Metal Ecotoxicity in Soils Based on Interpretation of a Standardized Batch Test With siMPle BLM Models

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There is a need for easy-to-perform methods for site-specific assessment of ecotoxicological risks at metal contaminated sites.

Soil extracts from standardized batch tests with dilute CaCl₂ performed to assess mobility of contaminants at contaminated sites has a potential to be used also to assess ecotoxicological risks. Such soil extracts could potentially give a measure of the concentration of metals in the soil solution that is available to soil organisms and plants, thereby including effects of soil properties and contaminant 'ageing'. We have investigated the possibility to use a 0.001 M CaCl₂ batch test (ISO 21268-2) combined with biotic ligand models (BLMs) for assessment of ecotoxicity in soils. Concentrations of free metal ions (Cu²⁺, Zn²⁺) in soil extracts obtained with the batch test were linked to responses in ecotoxicity tests (microbial processes, plants and invertebrates) previously performed on metal spiked soils. Batch tests were performed with archived soil materials that were spiked using the same protocol as in the original studies. Then, the EC_x values based on free metal concentrations in soil extracts were related to pH by linear regressions. The model performance was compared to effects in field-contaminated soils. Our results indicate a pH dependence of EC₅₀ values based on free metal ions in the soil extracts, especially for Cu²⁺ where R² ranged from 0.83 to 0.93. Correlations for Zn²⁺ had R² 0.39 to 0.92. Comparing effect concentrations based on pH adjusted Cu²⁺ and Zn²⁺ in soil extracts from spiked and field contaminated soil samples, the toxic responses were similar. This indicates a potential of the calibrated models to assess toxic effects in field-contaminated soils, taking differences in soil properties and effects of contaminant 'ageing' into account. Consequently, evaluation of standardized 0.001 M CaCl₂ batch tests with a simplified BLM can provide the basis for an easy-to-use tool for site specific risk assessment of metal ecotoxicity in soil. Such a tool would also be applicable to soils containing sorbents with properties that differ from natural soil components e.g ash, soot or amendment with biochars.

3.19.T-04 Assessing the Sensitivity of Benthic Macroinvertebrate Communities to Zinc in the Field

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Zinc is ubiquitous in the natural environment. The natural background level of zinc can be frequently enriched in downstream surface waters by anthropogenic activities that result in emissions from both controlled point and diffuse sources. Environmental Quality Standards (EQS) are typically derived from the results of laboratory studies on single species, and although there may be a significant number of individual species that are tested for zinc toxicity this will always be much more limited than the diversity of species that are found in real ecosystems. Furthermore, in these single species tests, usually only one stressor is being studied. Consequently, there is always some uncertainty surrounding the protectiveness of an EQS when it is applied to real ecosystems containing a multitude of chemical and physical stressors. A threshold derived based on the total abundance of eight sensitive taxa could potentially be used as an indicator of the overall ecosystem sensitivity. However, the inclusion of some responsive, but relatively insensitive taxa in this ecological response metric could bias the results from it towards a higher threshold. To assess this possibility, the least sensitive species were progressively removed from the collective ecological metric, basing the analysis on a progressively smaller number of the more sensitive species. There is a compromise between the robustness of the analysis and the sensitivity of the sub-community that the analysis is based on. Analyses that are based on fewer species or taxa may provide a more sensitive result, but are subject to greater levels of uncertainty because of the proportion of low exposure sites at which that none of the taxa that are included in the analysis are present due to the habitat being unsuitable. This approach was able to assess this real ecosystem data and evaluate the uncertainty associated with the protection provided by the EQS for zinc.

3.19.T-05 Deriving Nickel (Ni(II)) and Chromium (Cr(III)) Based Environmentally Safe Olivine Guidelines for Coastal Enhanced Silicate Weathering

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Active removal of 150 – 1180 gigatons of carbon dioxide (CO₂) from the atmosphere is needed to keep global warming below 1.5 °C. Negative emission technologies (NETs) such as coastal enhanced olivine weathering could help achieve this goal. Coastal enhanced olivine weathering aims to speed up the natural CO₂-consuming chemical weathering of the silicate mineral olivine (Mg₂xFe₂(1-x)SiO₄) by dispersing finely ground source rock in dynamic coastal waters. However, olivine contains nickel (Ni) and chromium (Cr), which may pose a risk to marine biota during a gigaton-scale coastal application. Currently, olivine toxicity data is lacking, but marine environmental quality standards (EQS) for Ni and Cr considered individually are available. Therefore, this study aimed to derive a maximum amount of olivine that could be dispersed in the coastal seas without exceedance of the Ni or Cr EQS. Calculations were made for the two limiting application scenarios in which all Ni and Cr released during olivine weathering end up in either the water column or the sediment. Results showed that metal concentrations in the water column are unlikely to exceed existing EQS due to the large seawater dilution effect. However, sedimentary metal concentrations could exceed EQS, and pose a potential risk to benthic marine biota, once application exceeds 1.4 kg of olivine m² of seabed. Accordingly, on a global scale a maximum of 39 Gt of olivine could be dispersed, and the equivalent amount of CO₂ could be sequestered during the 21st century. On the basis of current EQS, we conclude that adverse environmental impacts from Ni and Cr release could reduce the applicability of olivine in coastal enhanced silicate weathering. Our findings call for more in-depth studies on the potential toxicity of olivine toward benthic marine biota, especially in regard to bioavailability and metal mixture toxicity.

3.19 Trace element biogeochemistry, exposure and impacts in ecosystems (Part II)

3.19.T-06 Mercury in Terrestrial Vertebrates and Possible Biomagnification in Foodchains in Switzerland

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The majority of atmospheric mercury is estimated to be deposited in terrestrial environments, which becomes an important place of transfer and bioaccumulation in local food chains. The assessment values for mercury in Switzerland are available only for fish for human consumption and the international assessment values for mercury is available to protect top predators only in aquatic food chains. Mercury is toxic to organisms even in sublethal doses: it is persistent, bioaccumulates and biomagnifies. Hence, there is a need to define the environmental quality standard for meat from game for human consumption and to protect apex predators in terrestrial food chains. This study focuses on mercury concentrations measured in terrestrial vertebrates in Switzerland providing an evidence of bioaccumulation of mercury in terrestrial vertebrates, and aims to assess the possible biomagnification up the trophic level. Mercury was measured in the tissues samples (muscle, liver, kidney) of roe deer and eurasian lynx, and feathers of barn owls. Total mercury (T-Hg) was measured using Direct Mercury Analyser. Methylmercury (MeHg) was measured using HPLC-ICP-MS using a single step extraction in L-cysteine. Fresh weight T-Hg in roe deer muscle (n=81), liver (n=66) and kidney (n=68) ranged between 0.28-31.73 µg/kg, 0.73-78.39 µg/kg and 2.96-975.94 µg/kg respectively. Fresh weight T-Hg in lynx muscle (n=27), liver (n=29) and kidney (n=38) ranged between 2.74-194.39 µg/kg, 3.29-201.44 µg/kg and 3.43-423.01 µg/kg respectively. Dry weight T-Hg in down feathers of 12 barn owls ranged between 54.12-185.16 µg/kg. Levels measured above the limit of quantification of 0.03ng implies bioaccumulation in the terrestrial vertebrates in Switzerland. Significant difference ($p < 0.05$) was found in mean T-Hg concentration in muscle of roe deer (prey) and lynx (predator), with a biomagnification factor of 10. Hence, mercury could biomagnify up the trophic level also in the terrestrial food chain.

3.19.T-07 Titanium Dioxide Nanoparticles Modulate the Accumulation of Inorganic and Methyl Mercury by Crustacean *Daphnia magna*

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The overarching goal of the present study was to explore the role of nTiO₂ on the uptake and depuration of two major aquatic mercury species (inorganic, IHg and monomethyl mercury, MeHg) by *D. magna* through waterborne exposure and algal diets. To obtain an insight into the role of nTiO₂ on the accumulation of IHg/MeHg through water-, particle- and alga-borne exposures, *D. magna* was: (i) treated with mixtures of 2x10⁻⁸M IHg/MeHg and 20 or 200 mgL⁻¹nTiO₂; (ii) exposed to nTiO₂ pre-treated with IHg/MeHg; (iii) feed on alga *Chlamydomonas reinhardtii* pre-treated with IHg/MeHg; (in the absence and presence of nTiO₂). The results were compared with exposure of *D. magna* to dissolved IHg/MeHg alone. Experiments were performed in synthetic freshwater and in three independent replicates. nTiO₂ particles used in this study have a primary particle size of 5 nm, 100% anatase, purity 99.8%. *D. magna* was exposed for 2h to measure the intake of Hg, than transferred to clean standard freshwater without any food for depuration for 22h. Total accumulated Hg (THg=IHg+MeHg) in *D. magna* by atomic absorption spectrometry using the Advanced Hg Analyzer AMA 254 (Altec s.r.l., Czech Republic) and expressed in mg kg⁻¹ dry weight of biomass. The stability of nTiO₂ suspensions was also characterized. The results of the present study demonstrated the complex role of nTiO₂ played in the accumulation by zooplankton of two major Hg species present in the aquatic environment – IHg and MeHg. IHg has higher reactivity to nTiO₂, than MeHg, while MeHg has higher reactivity with living organisms, such as alga and daphnids. nTiO₂ played an important role, but dual role in the accumulation of both Hg species through the waterborne and foodborne exposure by reducing the assimilation efficiency for both IHg and MeHg through waterborne exposure, but increasing assimilation efficiency for MeHg when *D. magna* was fed on alga pre-treated with MeHg. nTiO₂ led to greater trophic transfer efficiency for both IHg and MeHg through feeding on TiO₂ pre-treated with IHg/MeHg, but lessened trophic transfer efficiency for both IHg and MeHg through feeding on alga pre-treated with Hg. The present results will help to better understand the role of nTiO₂ on bioavailability and trophic transfer of global contaminants, such as mercury, known to bioaccumulate and biomagnify in the aquatic environment.

3.19.T-08 Methylmercury Trophic Transfer: How Exposure Methods Affect Aquatic Invertebrate Biomagnification

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Representative laboratory studies of contaminant cycling depend on mimicking naturally relevant conditions in an unnatural setting. Some toxicants, like methylmercury (MeHg), are highly complex in their freshwater trophic transfer, with field studies documenting wide variability in biomagnification rates. Experimental work is essential to understand this variability, yet the efficacy of laboratory methods to characterize MeHg transfer in aquatic food webs remains questionable. Our work unpacks how different methodologies affect trophic transfer of MeHg from oligochaete prey (*Lumbriculus variegatus*) to larval dragonfly predators (Anisoptera sp) using three different methodologies. We quantified dragonfly dietary bioaccumulation from prey dosed aqueously with MeHg-chloride vs MeHg-cysteine, and with prey dosed dietarily with MeHg-contaminated algae. All experiments used 4 MeHg concentrations and ran for 5-8 weeks. We also evaluated impacts of exposure on dragonfly growth, body condition, feeding rate, predator avoidance behavior, and immune response. Preliminary data suggest that dragonflies bioaccumulate MeHg in a dose dependent manner but there is no difference between MeHg-chloride and MeHg-cysteine trophic transfer ($p > 0.5$, Welch's t-test). All dosed treatments showed biomagnification factors less than 3, decreasing with increasing mercury dose. Prey dosed dietarily with MeHg contaminated algae further refined our study by exploring how increasing exposure realism impacts biomagnification. Whether these data indicate constraints in laboratory methods testing or a realistic view of biomagnification patterns occurring in nature will be explored. We will also present results of a meta-analysis quantifying effect size and variability drivers in predatory invertebrate MeHg biomagnification reported in the literature. Environmentally representative laboratory methods are critical to accurately quantify contaminant risk and characterize toxicity, and successful future monitoring and modeling efforts depend on deeper examination of dietary accumulation by predatory invertebrates.

3.19.T-09 Improving Toxicokinetic-Toxicodynamic Models for Metals: Interspecific Variability of Mechanisms Within the Genus *Daphnia*

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To advance the ecological relevance of Environmental Risk Assessment (ERA) of metals, effects of metals and metal mixtures on the level of communities and ecosystems need to be understood, and should be predictable based on information from lower levels of organization. To this end, toxicokinetic-toxicodynamic (TKTD) models need to be developed for multiple species and metals to provide the link between individual-level single-metal and community-level combined effects. Dynamic Energy Budget (DEB) theory provides a framework that makes such models applicable in a range of ecological scenarios. A set of four DEB-based toxicodynamic mechanisms (costs for structure, maintenance costs, assimilation efficiency, reproduction efficiency), typically applied one-at-a-time, has been used to model toxicity of a variety of substances, including metals, to a variety of organisms. For metals, there is reason to expect that the necessity to deviate from this standard approach is the rule rather than the exception, due to their potentially wide range of effects on lower levels of organization. We conducted life-table experiments with three species of *Daphnia*, exposed singly to Cu, Ni and Zn and inferred TKTD mechanisms within an Individual-Based Modelling (IBM) approach, providing insight into interspecific variability in TKTD mechanisms of metals, and the potential need to widen the range of DEB-based mechanisms that need to be considered. We found considerable interspecific variability not only in terms of sensitivity, but also in terms of the quality of effects on reproduction, carapace length and survival. The standard approach (four basic mechanisms applied one-at-a-time) was sufficient to explain observed effects in some cases (e.g., effects of Ni and Zn to *D. magna*), but not in others (e.g., effects of Ni to *D. pulex*). In those cases, multiple mechanisms acting simultaneously, including stimulating effects, need to be assumed in order to explain observed effects. Our findings indicate limited generalizability of mechanistic insight gained from standard test species, even among closely related species, and highlights the relevance of natural diversity for ERA.

3.19.T-10 Importance of Including All Life Cycle Effects When Modelling Copper Effects on Brook Trout (*Salvelinus fontinalis*) Populations

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Population models can be helpful to predict population-level effects based on already available toxicity data on individual-level endpoints. For copper (Cu) effects on trout, most studies have been performed with early life stages. However, true population-level effects may be missed if the risk analysis is based solely on early-life stage effects. Therefore, the aim of this study was to predict population-level effects of Cu on a brook trout (*Salvelinus fontinalis*) population using a population model based on observed individual-level effects of a full life cycle study and an early-life stage study. We hypothesized that the population-level to individual-level effect concentration ratio would be smaller for predictions based on the life-cycle study than for predictions based on the early-life study. Population-level effects of Cu on a brook trout population were predicted with an Individual Based Model (i.e. inSTREAM-Gen) based on observed individual-level effects from either a full life cycle study or an early life stage study. Effects of Cu on individual-level endpoints were implemented based on relative effects compared to the control. For the life cycle study, we combined endpoints on survival, growth, reproduction, and development and for the early-life stage study, we implemented effects of hatching success, survival, and growth of yolk-sac larvae. Population-level NOEC (NOEC_{pop}) values were determined as the highest concentration for which no effect larger than 10% on trout density compared to the control was predicted. Furthermore, predictions based solely on the early-life stage effects of the full life-cycle study (hatching success, hatching time and survival and growth of yolk-sac larvae) were made. In addition, we assessed the effect of the implementation method of survival on the predicted NOEC_{pop}: Individual Tolerance (IT) vs Stochastic Death (SD). Overall, our results showed higher ratios of NOEC_{pop} to individual-level NOEC based on the early-life stage study (> 2.6 to 4.2) than for the life-cycle study (0.4 – 1.0). This was supported by simulations based solely on the early-life stage effects of the life-cycle study (ratios ? 3.4). This confirms our hypothesis that predictions of population-level effects could underestimate true population effects if solely based on early-life stage effects. Furthermore, we found that the implementation method of survival effects (IT or SD) is important for the predicted NOEC_{pop}.

3.19 Trace element biogeochemistry, exposure and impacts in ecosystems (Part III)

3.19.T-11 The Exposure and Fate of Platinum in the Freshwater Environment: An EcoToxicological Approach

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Platinum (Pt) is a precious metal with unique chemical and physical properties, that makes it indispensable. It is primarily used in automotive catalytic converters, from where it is emitted, but mining and chemical facilities are also primary sources of Pt into the environment. Extensive research has been conducted on the fate and effects of Pt entering the environment via road dust resulting from mechanical and chemical abrasion of catalytic converters. However, limited studies have been conducted on Pt exposure from mining and production activities, while data on the occurrence of Pt in biota near production sites, is still lacking. Therefore, the aims of the present study were to determine the Pt concentrations in the aquatic environment, establish the transportation and bioavailability of Pt in the aquatic system, and subsequently establish the aquatic biota community structures to determine the effects and potential risk of Pt to biota and humans. Field surveys were completed, where water, sediment, macroinvertebrate

samples, three different fish species, as well as their associated parasites were collected for metal analysis. In all of the samples metal(loid)s that are normally associated with Pt mining activities were targeted, thus concentrations of As, Cd, Cr, Cu, Pb, Ni, Pt and Zn were determined. Dissolved concentrations of As, Ni, Cu and Pt, as well as concentrations of all the targeted metal(loid)s in the sediment were significantly higher after the influence of intensive mining activities. Macroinvertebrate community structures were significantly altered by the mining activities and changed from moderately sensitive taxa at the reference site to highly tolerant taxa at the impacted site, while both diversity and functional approaches indicating metals as the main drivers in community changes. Concentrations of As, Cr and Pt were significantly higher in fish collected from the impacted impoundment compared to the reference impoundment, whereas their associated cestodes and nematodes accumulated non-essential metals more readily compared to their hosts. These high metal(loid) loads also pose several carcinogenic and non-carcinogenic risks to humans who consume these contaminated fish. The present study provides valuable and missing data on the transformation and bioaccumulation of Pt and associated metal(loid)s from mining and production activities, as well as the negative effects it has on the aquatic ecosystem.

3.19.T-12 Distribution of Rare Earth Elements in the Food Web of Estuarine Ecosystems: The Case Study of the Loire Estuary

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Rare earth elements (REEs) regroup 17 metallic elements including the fifteen lanthanoids (from lanthanum to lutetium), yttrium and scandium. Their increasing use in many industrial sectors causes an increase of discharges into the environment and in particular in estuarine areas subject to strong anthropogenic pressures. This study focused on assessing the distribution of REEs along the food web of the Loire estuary (France). Several species (*Heteromastus filiformis*, *Nephtys hombergii*, *Nereis diversicolor*, *Coruphium volutator*, *Limecola balthica*, *Scrobicularia plana*, *Chelon ramada* and *Anguilla anguilla*) representative of different levels of the Loire estuary food web were sampled on different sites and their REE concentration was measured. Intra-species differences have been highlighted with much greater concentrations of light rare earth elements (LREE) than of medium (MREE) and heavy (HREE) ones for all species. LREE contribution was around 71%, whereas they were only 13 and 16% for the MREE and HREE. In addition, a higher REE accumulation was observed in lower trophic level species compared to organisms belonging to higher trophic levels (up to two times higher), demonstrating inter-species variations. Total mean REE concentration values were the highest for *C. volutator* (15 µg/g dw) and the lowest for *A. anguilla* (0.1 µg/g dw). Inter-phylum disparities were also noticeable. Among annelids, *H. filiformis* showed much higher concentrations than the two other annelid species. Among the two vertebrates, *C. ramada* presented five times higher concentrations than *A. anguilla*. European shale normalization allowed to highlight gadolinium positive anomalies in the REE patterns in most of the studied species. Only *C. volutator* stood out with a really diverging REE pattern preventing anomalies discrimination.

3.19.T-13 Effects of Different Forms of Gadolinium on Early LIFE Stages of Atlantic Cod (*Gadus morhua*)

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The lanthanoid element gadolinium (Gd) is one of the rare earth elements (REY). Gd-chelate complexes are commonly used as contrast agents in medical diagnostics (gadolinium-based contrast agents; GBCA). Due to rapid and unmetabolized excretion and limited retention in wastewater treatment plants, GBCA are released into the aquatic environment. Here we studied the effects of different Gd compounds on early life stages of Atlantic cod (*Gadus morhua*), namely inorganic Gd as GdCl₃, and the contrast agent gadoterate (Gd-meglumin) obtained as pharmaceutical injection solution (Gd-Sol) and as off-the shelf chemical powder (Gd-Meg). Nominal exposure concentrations reached from 3 to 3000 µg L⁻¹. Our results show that, while GBCAs were fully soluble at all tested concentrations, GdCl₃ solubility was limited in seawater, with average dissolved Gd concentrations reaching only 122±48 µg L⁻¹ even at the two highest exposure concentrations. Despite the lower exposure concentrations, GdCl₃ was most available for uptake in both eggs and larvae. It was further the most toxic of the tested Gd forms, significantly reducing larvae viability and body area, and causing deformations at exposure concentrations >100 µg L⁻¹. At the highest exposure concentration (3000 µg L⁻¹) Gd-Meg caused deformation phenotypes similar to GdCl₃, i.e., larvae with craniofacial deformities, lordosis and reduced pigmentation. In contrast, Gd-Sol did not cause significant adverse effects, likely due to effective protective effects from the chelating agent. This highlights the needs to study the long-term stability of GBCA-complexes in the marine environment.

3.19.T-14 Probing the Role of Metal Speciation in Copper Nanoparticles As a Driver of Adverse Outcomes During Embryogenesis of Urchins *Paracentrotus lividus*, *Arbacia lixula* and *Sphaerechinus granularis*

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Copper and copper oxide nanoparticles (CuNPs) are widely used in a broad range of industrial applications encompassing biotechnology, medicine and health care, biomonitoring and environmental remediation. Their use in ship anti-fouling paints presents a direct route into marine environments where they can impact on a wide range of organisms at all life stages, from embryos to adults. Sea urchins have been widely used as a model in developmental biology and as a bioindicator for the marine environment. In this study, the effects of copper and copper oxide nanoparticles (CuO, Cu₂O) on fertilisation success and transmissible damage to offspring during embryogenesis of the sea urchins *Paracentrotus lividus*, *Arbacia lixula* and *Sphaerechinus granularis* were analysed. Subsequently, the ability of the exposed sperm to successfully fertilise untreated eggs, given as fertilization rate, was tested. After fertilisation, zygote growth to the pluteus larva stage was monitored over 72-96 h and these offspring of treated sperm were scored for developmental defects, developmental delays and death. While copper ions are

known to be toxic to organisms and are often used as a positive control in bioassays, copper and copper oxide nanoparticles showed values close to those of Cu²⁺ ions. Furthermore, zero-valent copper nanoparticles were found to be least toxic while the oxides of copper caused greater suppression of fertilisation. All plutei offspring showed increasing numbers and types of developmental defects as a function of copper concentration. The most common abnormalities were found to be delayed development, exogastrulation and skeletal defects and were present in plutei of all three urchin species. Exposure of sperm to copper nanoparticles may have significant negative consequences not only in their ability to successfully fertilise eggs but also in potentially causing transmissible damage to the subsequent offspring. Ultimately, the species of urchin must also be considered when investigating the toxicity of nanoparticles since not all species are equally sensitive.

3.19.T-15 Integrated Exposure and Effects Assessment of Three Trace Metals on a Marine Copepod *Tisbe battagliai*

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Some trace metals at low concentrations are essential for living organisms, yet they are environmentally ubiquitous and may bioaccumulate to become toxic at higher concentrations. Increasing coastal industrial development is driving a rise in effluent discharge (including metals) into coastal waters. These metals can exist as complex chemical mixtures, which may cause greater toxicity than predicted from single data. Metal speciation influences metal toxicity, as it can affect their bioavailability and uptake. An integrated exposure and effects assessment was conducted for a combined three metal study on an ecologically important coastal copepod *Tisbe battagliai*. Copper, nickel, and zinc are among the most common metals associated with toxicity in marine environments, making them good candidates for cumulative effects assessment. The experimental design used was Definitive Screening Design (DSD) that can determine main effect drivers and second-order effects. DSD uses LC₁₀, LC₅₀ and LC₈₀ values from single metal exposures to design 15 combined solutions. This study aimed to: (1) characterise the metal exposure solutions by size fraction; and (2) complete an acute, 24-hour combined effects assessment focusing on the metal-induced reactive oxygen species (ROS) – DNA damage – apoptosis toxicity pathway. This was achieved by measuring relevant endpoints and conducting size fractionation to determine each metal fraction's (particles, colloids, and low molecular mass – LMM) contribution to total concentration. Reduced survival after 24 hours was driven by copper, nickel, and zinc in the mixture, with significant interactions between copper*zinc and copper*nickel (P < 0.05). For cellular ROS formation and oxidative DNA damage, no particular metal was the main toxicity driver. For oxidative DNA damage, all paired metal combinations within the three-metal mixture had non-parallel interaction profiles, showing that the effect of each metal on DNA damage changed as other metal concentrations changed. Combined metal solutions were dominated by nickel in the LMM form, which is the most bioavailable, whereas copper and zinc were present in all three fractions. Copper was the only metal that did not have the highest contribution from LMM but remained a main effect driver for survival. An integrated exposure and effects approach allowed us to use metal fractionation data and interactions between copper, nickel, and zinc to better understand the observed toxicity.

3.19 Trace element biogeochemistry, exposure and impacts in ecosystems (Virtual Only)

3.19.V-01 Disclosing Short Time Scale Toxicokinetics and Toxicodynamics of Inorganic Mercury in the Saltmarsh Plant *Halimione portulacoides*

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Saltmarsh plants as *Halimione portulacoides* have a key role on the mercury (Hg) biogeochemical cycle, namely by accumulating high levels of this metal in the roots. Despite that, research on Hg toxicokinetics (uptake, accumulation, elimination) in those plants is scarce, while there is still a lack of knowledge on related toxicodynamics comprising biochemical responses. A short-term exposure (4 h; under hydroponic conditions) of *H. portulacoides* to realistic levels of inorganic Hg (iHg) (0.45 µg/L) was carried out, under light and dark conditions in order to clarify the influence of light-dependent activity on iHg toxicokinetics and toxicodynamics. Plants were collected in a coastal lagoon (Aveiro Lagoon, Portugal), namely at a highly contaminated site (Laranjo - LAR) and a reference site (REF), allowing an evaluation of the contamination background interference. Plants were acclimated to the lab for two months before exposure. Samples of leaves and roots were collected after 2 h (T2) and 4 h (T4) of exposure, as well as immediately before exposure (T0) for analysis of total Hg levels, antioxidant defences [catalase (CAT), ascorbate peroxidase (AP), glutathione peroxidase (GP), superoxide dismutase (SOD)] and oxidative damage [lipid peroxidation (LPO)]. Total Hg levels in the roots of *H. portulacoides* of both sites were similar at T0, suggesting elimination during the acclimation period and allowing a straightforward comparison of plant responses to the current Hg exposure. At T4, an enhancement of total Hg levels was recorded in roots of exposed plants from LAR at daylight, while at dark conditions no significant accumulation was found. No changes on antioxidants activities or LPO were recorded in LAR plant roots in T4 at daylight, suggesting a tolerance to Hg accumulation. An opposite pattern was found for REF plants at T4 with no significant Hg accumulation at daylight and a notorious enhancement in dark conditions. A concomitant decrease of AP activity was recorded in the roots of plants from REF. In general, no significant increases of Hg levels were found in the leaves of plants from both sites over time. Overall, the results highlight the rapid uptake of iHg by *H. portulacoides* roots (within 4 h) and a possible role of the plant Hg historical background on the toxicokinetics and toxicodynamics of iHg. Naïve plants seem to be more vulnerable to iHg toxicity than those from a chronically contaminated site.

3.19.V-02 Ecotoxicity and Human Risk Assessment of an Anthropogenic Contaminated Site

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One of the most common anthropogenically contaminated sites in developing country is landfill area. The pressure of urbanization has led to increased waste generation in major cities which are commonly deposited in landfill; hence, heavy metals contaminations are closer to human environment more than ever. The study examined the Ecotoxicity of Arsenic (As), Cadmium (Cd), Zinc (Zn), Manganese (Mn), Copper (Cu), Cobalt (Co), Chromium (Cr), Nickel (Ni) and Lead (Pb) and health-related risk from human activity of waste disposal using the ISSO methodology standard. The finding indicated that the concentration of the elements descended as follow; Mn>Zn>Cr>Pb>Cu>Ni>Co>As>Cd where all the element concentrations exceeded the WHO permissible limit. The Enrichment factor (EF) and potential ecological risk (ER) indicated no enrichment and low risk for all the elements except Cd (3.67, 426) with moderate enrichment and very high risk. The hazard index (HI) value of the elements indicated no significant risk of non-carcinogenic effect for both Adult and Children except for Mn in Children with value of 1.69×10^0 ; however the HI value of the elements descended as; Co>Mn>Zn>As>Cd>Cu>Pb>Ni>Cr for adult and Mn>Cd>Co>Cr>As>Zn>Pb>Cu>Ni for children. The total carcinogenic risk (TCR) value of the toxic metals descended as Ni>Cd>Cr>As>Pb for both Adult and Children while the metals showed no carcinogenic to acceptable risk to humans. Improving the method of waste disposal and management is needed to limit the potential human health risk and environmental contamination. Further studies on the bioaccumulation and availability to surrounding edible crops/plants are needed to fully understand the environmental impact of the anthropogenic contamination action on plant.

3.19.V-03 Ecotoxicological Implications Due to Chronic Exposure to Relevant Concentrations of Rare Earth Elements

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Rare Earth Elements (REE) are not as sparse in the environment as their name suggests. Currently, they play an essential role in a number of technological applications from electronics and fuel additives to agriculture and even environmental remediation. REE production spiked especially after 2010, and worldwide REEs anomalies have been observed in sediments, rivers, and lakes, estuaries and coastal waters, ground water and tap water as well as in aquatic species and humans. However, the potentially toxic effects resulting from exposure to REE in human health and the environment biota are not currently under the radar of lawmakers that legislate environmental quality standards for chemicals and for all ground and surface waters, where most of the legal efforts are focused on mine industry of these elements. This is also not helped by the existence of a generalized gap of synthesized information about REE environmental behaviors and impacts associated with biological effects including both Human and other animal species, especially considering more realistic scenarios and its ecological implications. REEs have been reported to induce neurotoxicity and oxidative damage in the central nervous system. However, due to limited underpinning experimental and field data, such effects are poorly documented. In this study, chronic waterborne exposure of zebrafish (*Danio rerio*) to two REE (Lanthanum (La) and Gadolinium (Gd)) at relevant concentrations altered fish key survival behaviors, such as social interactions dysfunction in shoaling formation. Furthermore, a clear unbalance between prooxidants and antioxidants was observed as a significant increase of oxidative damage and depleted defence activities, especially for La exposure. Metabolomics analysis of multiple fish neurochemical signaling profiles are also presented. However, despite the observed effects, the detection of these elements in fish tissues was indetermined.

3.19.V-04 Export of Mercury From Saltmarsh Vegetated Sediments During Flooding Period

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Several recent works indicated the elevated mercury (Hg) concentrations in saltmarsh sediments and its accumulation in the root-sediment system. During tidal flooding, the cycling of Hg between sediments and the water column is affected. To gain a better understanding of the export of Hg as well as the effect of water level fluctuations on the sediment-water exchange, overlying water (OW) and sediment cores (10-cm long) were sampled several times during the first 30 minutes of tidal flooding in one Hg contaminated saltmarsh in Ria de Aveiro. Immediately after sampling, OW were filtered and sediment cores sliced and stored in decontaminated plastic bags and centrifuged flasks. In the lab, separations were performed to obtain subsamples of sediments, pore waters (PW), and roots. Concentrations of reactive Hg [RHg] and total Hg [THg] were quantified in water samples and [THg] in solid sediments and belowground biomass. Physico-chemical parameters such as pH, temperature, salinity and conductivity were also determined in OW samples and humidity and organic matter (OM) in sediments. Dissolved [RHg] in OW increase in the first 4 minutes of tidal forcing due to flushing phenomena agreeing with the decrease of [RHg] in PW of all sediment layers from 0 to 2 minutes, suggesting a remobilization of Hg in the sediment. Overall [RHg] in PW increases on top layers after 2 minutes until it decreases significantly in the last 15 minutes, while in OW has a constant increase in the same time range implying a rapid export of Hg from sediments to the water column. Before flooding, [THg] in roots ranged between 9.34 mg/L (4-6cm) and 14.30 mg/L (6-8cm) in bottom layers, and increased to 16.42 mg/L (4-6cm) and 20.72 mg/L (6-8cm) in the first 2 minutes of inundation. The inlet of water that comes with the flooding increases the Hg concentration due to remobilization within the sediment. In the last 15 minutes, [THg] decreases in all root layers, which may suggest mobilization of Hg from the roots to the OW. Two peaks of [THg] were observed in roots and sediments in times 2 (20.7 mg/L in layer 6-8cm) and 15 minutes

(21.2 mg/L in 4-6cm layer), indicating a periodic trend for Hg remobilization between roots and sediments. The short-time variations registered in this study indicate a shift on the Hg equilibrium between PW, solid sediments and root surface. Moreover, as water floods the saltmarsh, Hg escape from sediments being potentially available to biota living in these ecosystems.

3.19.V-05 Long-Term Effects of Inorganic Mercury and Methylmercury in Naïve and Historically Exposed Saltmarsh Plants

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Following the Minamata Convention on mercury (Hg), worldwide efforts to appropriately characterize and manage related contamination have been raising. Wetlands provide several highly relevant ecosystem services, including the regulation of biogeochemical cycles. Despite this relevance and the frequent contamination-driven threats to these ecosystems, much is still unknown in some fields such as the responses of salt-marsh plants to Hg species while those have a major role in their sequestration and cycling. This study aimed to assess the oxidative stress responses and photosynthetic activity of a salt-marsh plant (*Halimione portulacoides*) to inorganic divalent Hg (iHg) and monomethylmercury (MMHg), comparing between historically exposed and naïve plants to appraise putative adaptation mechanisms. Plants collected in one contaminated site and one reference site, both permanently under tidal influence, were kept under hydroponic conditions for exposure to environmentally relevant concentrations of iHg or MMHg and a blank control for 14 days, under different salinity levels (0 and 21 PSU). The activity of catalase (CAT), ascorbate peroxidase (AP), glutathione peroxidase (GP), superoxide dismutase (SOD), as well as the production of thiobarbituric acid reactive substances (TBARS) as a result of lipid peroxidation (LPO), was assessed in roots and leaves. Salinity levels have little to no interference in the responses of the leaves to iHg and MMHg exposure, while enzymatic activities and peroxidative damage tend to be higher in roots exposed under low salinity levels. No significant alterations in the photosynthetic activity of leaves were found. Previous adaptation to the metal contaminants apparently constrains the oxidative stress responses of both leaves and roots, but patterns are dependent on the endpoint and the Hg form. In spite of these differential responses, significant effects of iHg or MMHg dosed at environmentally relevant concentrations were not always identified. This suggests that large plasticity ranges are consistent with previous evidence on the potential of *H. portulacoides* to tolerate Hg, accumulate it, and drive important phases of its biogeochemical cycle, which should allow the plant to cope with possible future scenarios of change in tidal dynamics.

3.19.V-06 Rhodium Speciation Analysis in Environmental Matrices Combining Different Analytical Techniques

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Rhodium (Rh) is one of the platinum-group elements less-studied in the environment due to its low abundance, irrespective of its steadily increasing concentrations. Anthropogenic sources are responsible for releasing Rh to all environmental compartments, whose main contributions are from automotive catalytic converters and industrial catalysts. The forms in which Rh is released are not yet entirely elucidated, and analytical challenges in Rh determination make its speciation analysis and environmental fate poorly known. This study addresses the existence and discrimination of soluble/insoluble Rh species in the operationally dissolved phase (< 0.45 µm) and its particulate fraction as well, using a combination of analytical techniques. To this goal, leachates obtained from urban road dust experiments and water samples from an estuary were investigated in order to elaborate Rh speciation analysis schemes. In filtered (< 0.45 µm) solutions, concentrations of truly dissolved Rh were measured by adsorptive cathodic stripping voltammetry (AdCSV) using a second derivative transformation of the voltammograms to improve Rh ultra-trace signal. In the same solutions, Rh total concentrations (truly dissolved + nanoparticles) were determined by inductively coupled plasma mass spectrometry (ICP-MS). Total concentrations of Rh in the solid samples were determined by AdCSV after an acid-digestion step with *aqua regia*. In the road dust leachates truly dissolved species of Rh corresponded to a considerable fraction of total Rh (~40 %), resulting from the compounds already in the oxidized form present in the road dust particles. The % of Rh dissolution in the leachates was higher when determined by ICP-MS as compared with those measured by AdCSV. This confirms the presence of different Rh forms, i.e. colloidal species and/or (nano)particles, measured by ICP-MS besides the truly dissolved forms (AdCSV). Despite the success of this strategy with urban road dust leachates, Rh determination by ICP-MS in waters, especially saline, is still challenging due to interferences. The improvement and combination of analytical strategies, sensitive enough to tackle elements at ultra-trace concentrations, may provide further insight into the speciation analysis of other platinum-group elements, e.g. palladium. *Acknowledgement* - Project IST-1018P.04037.1.01.; FCT funding SFRH/BD/111087/2015, PTDC/REQ- EPR/1249/2014, CQE UIDB/00100/2020-UIDP/00100/2020; COST Action TD1407 NOTICE.

3.19 Trace element biogeochemistry, exposure and impacts in ecosystems (Poster)

3.19.P-We136 Total Mercury, Methylmercury, Phosphate, and Sulfate Inputs to a Bog Ecosystem From Herring Gull (*Larus smithsonianus*) Guano

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Methylmercury is a form of mercury which is available for uptake by biota. It can magnify through trophic levels and leads to greater quantities within top consumers. The area of study, Brier Island Nova Scotia, receives upwards of 6000 herring gulls each

year in the spring during their nesting season. Previous research suggests that methylmercury hot spots are co-located with the highest density of the herring gull colony. This biovector mechanism has the potential to drastically alter the ecosystem through guano inputs. Samples were collected to analyze the temporal distribution of methylmercury within the guano. Samples were collected by installing five posts in the most populated area of the bog for birds to perch and excrete on. Collection of samples occurred every 2 weeks in the summer of 2018 and 2019 with a total of 43 samples. They were then taken back to Acadia University to measure total mercury (THg), methylmercury (MeHg), water-extractable phosphorous (PO_4^{3-}) and water-extractable sulphate (SO_4^{2-}) concentrations. THg concentrations significantly decreased throughout the nesting season (mean = 113.77 ± 50.22 ng/g, n=43) while %MeHg significantly increased (mean = 53.06 ± 24.94 %, n=40). This suggests a dietary switch because of gull nutritional needs or availability varies. PO_4^{3-} increased throughout the summer months (ranging from 2.8% to 4.4% of dry weight). Results suggested a variation in SO_4^{2-} as well (ranging from 0.1% to 0.8% of dry weight). These results are likely an underestimate as much higher concentrations can be found in feathers and carcasses. These data indicate that gulls are transporting considerable amounts of %MeHg, PO_4^{3-} and likely other contaminants that can alter environmental conditions of Big Meadow Bog.

3.19.P-We137 Examining Export and Bioaccumulation of Methyl Mercury in a Bog Habitat Impacted by Herring Gull Guano and WATER Table Restoration on Brier Island, Digby County, Nova Scotia

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The processes controlling Hg accumulation in bogs and the confounding effects of both gull guano input and increased flooding are not well known. Big Meadow Bog (Brier Island, NS) underwent water table restoration which was completed in the summer of 2018. The bog is also host to 4000-6000 herring gulls clustered in a small area in the Northern Bog that feed at nearby mink farming and aquaculture sites. Previous research in our group has shown that bird biomass and waste products are increasing the trace metals and nutrients measured in groundwater. Gull guano analysis results have shown that gull guano is very high in water soluble PO_4^{3-} and that total mercury (THg) and methylmercury (MeHg) are low in guano. Research from various studies examining terrestrial freshwater catchment flooding indicated that export of methylmercury (MeHg) increases 3-6 times over a 3–9-year time span in otherwise undisturbed catchments. An examination of water quality and lower trophic level biota was initiated to set a baseline for methylmercury concentrations in the base of the food web. Outflow water samples showed a seasonal trend between 2018-2021 with highest concentrations and % MeHg occurring during the summer period for 2019 and remaining lower for 2020-2021 data. The % MeHg increases to >60% in July of 2019 and reduces to < 40% in the following years. Similar patterns were observed in DOC (mid summer highs of 40-60 ppm DOC) and total phosphorus (0.5-2 mg/L) with a large portion present as orthophosphate. A survey of invertebrate bioindicators of mercury bioaccumulation in the food web indicated a wide range in concentrations (~0.06 - 1778 ng/g MeHg dry weight). Highest median MeHg concentrations were observed in water striders, water boatmen, milky backswimmers. Other invertebrates also showed high maximum MeHg concentrations in some individuals such as ground beetles (max 721 ng/g), midges (705 ng/g), spiders (1779 ng/g) and predaceous diving beetles (1074 ng/g). An examination of MeHg versus THg results for a subset of samples shows 4 – 133% of the THg is present in a MeHg form. The top 3 families for MeHg content (Corixidae, Gerridae, Notonectidae) have >90% MeHg likely due to feeding ecology and specific habitat chemistry.

3.19.P-We138 Total Mercury Concentrations in Vulnerable Species Along the South African Coast: Sharks, Skates and Rays As CASE Study

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A global decline in elasmobranch populations resulting from targeted fisheries or overfishing via by-catch raises conservation concern. In addition to these stressors, apex predators like sharks bioaccumulate high concentrations of total mercury (THg), due to biomagnification through the food chain. Although South Africa is considered one of the top ten contributors of Hg emissions globally, information on Hg concentrations in elasmobranchs is insufficient. The aim of the present study was therefore to evaluate the THg concentrations in elasmobranchs along the coast of South Africa. Total mercury concentrations were determined in 22 species of elasmobranchs (16 shark, 2 skate and 4 ray species) consisting of 86 individuals. Six shark and two skate species were collected from the south coast, while 10 shark and four ray species were sampled from the east coast. Concentrations ranged between 0.22 mg/kg THg in *Haplobpharus pictus* (dark shyshark) and 5.8 mg/kg THg in *Rostroraja alba* (white skate) on the south coast, whereas on the east coast it ranged between 0.21 mg/kg THg in *Mobula kuhlii* (shortfin devil ray) and 17.8 mg/kg THg in *Sphyrna lewini* (scalloped hammerhead). Total Hg on the east coast from the present study were in the same range or higher compared to the same species sampled on the east coast of South Africa from 2005–2010. The results from the present study were also compared to THg concentrations from the same species sampled in other countries, with generally higher concentrations along the South African coast. This indicate an on-going cause of concern for the health of these vulnerable shark species along the South African coastline.

3.19.P-We139 Mercury Photochemistry and Speciation in the Jijuktu'kwejk (Cornwallis River) Estuary of the Minas Basin, Nova Scotia: Effects of Salinity/DOM Interactions and Tide

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The photochemistry and speciation of mercury (Hg) are important factors in the retention of Hg in estuarine ecosystems. Net photoreduction of divalent Hg (Hg(II)) and volatilization of photoreduction products (elemental Hg (Hg(0))/dissolved gaseous Hg (DGM)) can be a mechanism by which Hg burdens in ecosystems are decreased. Using natural surface water, the effects of

salinity were investigated while controlling the concentration of high molecular weight (>1 kDa) DOM through a tangential ultrafiltration-dilution technique, and Hg photoreduction was quantified. Surface water was collected every 0.5 hours during a daylight semi-diurnal tidal cycle in the Jijuktu'kwejk (Cornwallis River). Net Hg(0) was measured in unfiltered surface water while total mercury (THg), easily reducible Hg (ERM; in this study an estimation of photoreducible Hg in filtered water), and DOM were measured in filtered surface water. In lab experiments, pseudo first-order rate constants in estuarine dilutions ranged between 0.22 h⁻¹ and 0.73 h⁻¹. The amount of Hg available for photoreduction in the dilutions (Hg(II)_{RED}) ranged between 67.25 pg and 265.91 pg (~336–1329 pg L⁻¹). Gross photoreduction rate constants decreased with increasing salinity and lower salinities (< 13.5 g L⁻¹) incurred the most drastic change in rate constant. In addition, DOM concentration decreased after irradiation (>57% loss) in lower salinity dilutions (? 13.5 g L⁻¹), suggesting a loss of DOM reactivity at lower salinities. In natural surface water, concentrations of TSS followed a pattern related to tide height with lowest concentrations observed during the periods of high and low tide. In unfiltered surface water samples, Hg(0) concentration was mostly nondetectable (< 10 pg L⁻¹) throughout the day. In filtered water, approximate Hg(II)_{RED} concentrations (measured from ERM analysis) followed an inverse pattern to the tidal height with a maximum concentration of 381 pg L⁻¹. While ERM measurements may overestimate the photoreducible Hg(II) fraction, Hg(II) photoreduction is likely suppressed in the study system due to high TSS loads greatly limiting the amount of radiation penetration in the water column. This research demonstrates the importance of salinity and DOM interactions as well as estuarine processes in influencing Hg photoreactions, as well as the importance of complementary laboratory and field measurements in mercury photochemistry research.

3.19.P-We140 Release of Indium From In₂O₃ Nanoparticles

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In recent years, the use and demand for indium in technology industries around the world has increased dramatically. This metal is a Technology Critical Element, TCE, widely used in the production of Indium Oxide (In₂O₃) and ITO (Indium Tin Oxide) for the manufacture of electronic devices such as electrically conductive transparent thin films in LCD's displays and photovoltaic cells. These materials, sooner or later, will reach the different environmental compartments, like freshwaters, but their behaviour and fate is not yet sufficiently known. Therefore, it is timely to study the dissolution process of In₂O₃ nanoparticles, in order to understand the release of free indium into the environment. This can be suitably achieved by using the electroanalytical technique AGNES (Absence of Gradients and Nernstian Equilibrium Stripping). AGNES has been developed to determine the free concentration of metals such as Zn, Cd, Pb, Sn or In in aqueous samples. In the case of nanoparticles, there is no need of any previous separation from their dispersions. The general protocols for working with indium, such as the determination of the preconcentration factor or gain by calibration, have been developed in recent works. In addition, the use of the rotating disk electrode effectively reduces the required metal deposition times. Solutions of KNO₃ at different pH values and synthetic seawater (at pH 8.0) were prepared and put in contact with In₂O₃ nanoparticles and bulk In₂O₃ for months maintaining constant temperature and agitation. AGNES provided the dissolution curves, from whose final equilibrium concentrations the values of the solubility constants of these materials can be obtained. *Acknowledgement* - Support from the Spanish Ministry of Science and Innovation (Project PID2019-107033GB funded by MCIN/AEI/10.13039/501100011033) and from FISDUR-Generalitat de Catalunya (KRS).

3.19.P-We141 Influence of Organic, Inorganic and Absence of Phosphate on the Toxicity of Lanthanum and Gadolinium to *R. Subcapitata*

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Lanthanides (Ln) are a group of 15 metals, ranging from lanthanum to lutetium. These elements are widely used because of their unique properties in modern technologies such as for electric cars, batteries, lasers, and smartphones. This has led to an increased risk for the environment. Lanthanides are now considered as emergent pollutants and studies of their impact on ecosystems has become an urgent issue. However, Ln toxicity experiments remain complicated due to the potential precipitation or complexation of these metals. This attributes to a strong affinity with several ligands such as phosphate. Phosphate is an essential element for algal growth and can easily interact with Ln. In the current study the impact of phosphate on La and Gd toxicity on the freshwater microalgae *Raphidocelis subcapitata* has been assessed. Four different test media were compared; (i) DIN media from the international guideline ISO (2012) containing inorganic phosphate, and two modified DIN media where phosphate was replaced with two different organic phosphates: (ii) glucose-1 phosphate (DIN-G1P) and (iii) cyclic adenosine monophosphate (DIN cAMP); (iv) and a fourth media without any phosphate (MHSM). *R. subcapitata* was exposed to La and Gd at five different nominal concentrations (0.01; 0.1; 1; 10 and 100 mg/L) for 72h. Growth inhibition was measured and EC50 values were compared for all media. According to first results, the presence of inorganic phosphate led to severe precipitation and complexation. For Gd, the EC50 measured for DIN was significantly lower than the EC50 measured for DIN-G1P and DIN cAMP. Notable is that the EC50 measured in the media with absence of phosphate (MHSM) was found to be lower than in the DIN media, but higher than the two media with organic phosphate (EC(DIN-G1P), EC(DIN cAMP) < EC(MHSM) < EC(DIN)). The lower toxicity of MHSM did not comply with our hypothesis, but could be explained by the incapacity of *R. subcapitata* to grow correctly in this media limiting the growth inhibition analysis. The first results also indicate that with La, toxicity seems to be significantly different to Gd. La tend to precipitate strongly which could explain why the EC50 measured were significantly higher than for Gd. More detailed toxicity and speciation results will be presented in the poster.

3.19.P-We142 The Influence of Sediment Geochemistry on Methylmercury Bioaccumulation in Coastal Invertebrates in the Minas Basin, Bay of Fundy

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Methylmercury (MeHg) is a toxic contaminant that readily bioaccumulates and biomagnifies in food webs, and negatively affects organism and ecosystem health. Impacts on ecosystem health from MeHg have been extensively studied in freshwater ecosystems however much less is known about MeHg retention and biomagnification in coastal ecosystems. Intertidal invertebrates are abundant in estuaries and are critical prey sources for migratory birds and marine fish, and thus determining the uptake of MeHg by intertidal invertebrates is essential for determining MeHg exposure in higher trophic level organisms in the food web. This research quantifies MeHg levels in invertebrates and relates bioaccumulation of MeHg to changes in sediment porewater concentrations of sulfate, dissolved organic matter, and mercury in five coastal estuarine locations in the Minas Basin, Bay of Fundy. The formation of MeHg by sulfate-reducing bacteria during the reduction of sulfate to sulfide suggests that systems with sulfate loading may have increased MeHg concentrations in sediments. However, binding of Hg with sulfide may reduce bioavailability of these complexes to methylating bacteria. Dissolved organic matter (DOM) may reduce the uptake of MeHg by invertebrates due to its size, however DOM may also increase MeHg production by acting as an energy source for methylating bacteria. To assess the effects of sediment geochemistry on MeHg bioavailability to invertebrates, organism MeHg concentrations were compared to total organic carbon (TOC), sulfur speciation, and mercury speciation in pore water and sediment. We found that bioaccumulation of MeHg in invertebrates (with a focus on *Corophium volutator*, *Ilyanassa obsoleta*, and Polychaete spp.) was not influenced by total mercury levels in sediments, or sulfate concentrations in pore water. However, a positive correlation between sediment total carbon content (measured as % Loss on Ignition) and total mercury was found. This research provides quantitative data on MeHg bioavailability in the Minas Basin which can be used to protect both ecosystem and human health. By identifying areas that are at greater risk for increased MeHg production this research sheds new insight on the health of ecosystems critical to migratory birds, coastal fisheries, and many industries in Atlantic Canada.

3.19.P-We143 Environmental Risks of Tungsten-Based Nanoparticles to Freshwater Ecosystem Using Indoor Aquatic Mesocosms

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Tungsten (W) is a transition metal that has commonly been used in many industrial, and scientific, applications due to its unique physical and chemical properties (Koutsospyros *et al.*, 2006). Actual applications include the use of **tungsten nanoparticles** in different fields as wastewater treatment, hydrogen storage, biomedicine, as well as in solar cell and nuclear fusion reactors (ITER project). Due to its widespread use and its potential incidental release, the environmental exposure and hazards remains to be further understood (Strigul *et al.*, 2009). The aim of our works is to assess the environmental risk of W and WO₃ nanoparticles towards freshwater ecosystem, using indoor aquatic mesocosms which are considered as a robust experimental design to study the exposure and impact of nanomaterials. Experiments were conducted in twelve 16 L-mesocosms simulating a lentic ecosystem represented by natural picoplanktonic and picobenthic communities, one macro algae (*Chara vulgaris*, one macrophyte (*Ranunculus aquatilis*) and one benthic grazer (*Physella acuta*). A mesocosm is defined here as an enclosed and essentially self-sufficient (but not necessarily isolated) experimental environment or ecosystem with a number of interdependent system parameters. Tanks were chronically contaminated with the W and WO₃ nanoparticles during 4 weeks to reach nominal concentration of 2.5 m².L⁻¹ at the end of the experiment. The (bio)distribution and (bio)transformation were assessed by inductively coupled mass spectrometry and X-ray absorption spectroscopy. The biological impacts on the organisms were studied in term of mortality, growth and development. These results will be discussed in term of speciation-dependent biological effects of W-based nanoparticles, and trophic transfers under a realistic exposure scenario (chronic low-level additions of W, mid-term exposure).

3.19.P-We144 Platinum Partitioning and Effects in Aquatic Microcosms

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Platinum is increasing in aquatic environments and therefore poses a risk to biological communities. Research studies (laboratory and field based) have indicated platinum bioavailability to biological communities is dependent on partitioning within environmental compartments. Therefore, the aim of this study was to investigate platinum partitioning and effects in semi-controlled aquatic microcosms. Microcosms were established for two months at the North-West University METSI facility. Following the establishment period, microcosms were exposed to a range of standard platinum concentrations (0.1, 1, 10, 100, and 500 µg/L) for eight weeks. Samples were collected at 48 hours, four weeks, and eight weeks. Water, sediment, macrophytes and artificial mussel samples were analysed for platinum. Platinum were quantified using an Atomic Absorption Spectrometer (AAS) and an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) to determine the different concentrations within each of the compartments. Platinum concentrations in the water column decreased over time, whereas the sediment, macrophytes and, artificial mussels (AM) showed higher initial accumulated platinum followed by a progressive decrease over time. The accumulation levels between the two macrophyte species varied, and *Frontalis* sp. had a higher accumulation level than the *Charophyta* sp. Diatom community assemblages were sampled at 48-hours (initial) and eight weeks (chronic) after exposure.

Initial samples (48 hours) indicated diatom communities showed little to no response; however, all the concentrations showed the presence of pollution tolerant taxa after the eight-week exposure. Diatom deformities were also observed at platinum levels higher than those present in the environment (500 µg/L). A total of 25 type 1 teratologies were identified from four 500 µg/L chronic exposure samples. Resulting in 1.025% of teratologies identified for the epiphytic diatom community and 1.875% of teratologies for the benthic diatom community. This teratology indicates a possible disruptive potential of increased platinum concentrations on aquatic communities. Overall, this study provided information that forms a valuable link between existing laboratory based platinum studies and various field based studies that have been implemented in South Africa. It is recommended to further investigate platinum effects in aquatic communities and to determine if teratologies seen in diatoms are found in field collected samples.

3.19.P-We145 The Use of Passive Samplers and Native Organisms to Monitor Platinum Exposure in a Freshwater Environment

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The artificial mussel (AM) is a passive sampling device that was originally developed for monitoring metal concentrations in the marine environment, but is also increasingly used in freshwater environments. The AM allows the determination of the dissolved, bioaccessible metal fraction in water bodies without killing organisms, as well as environments with unfavourable conditions for living bioindicators. The AM consists of a non-permeable Perspex tube, which is closed on both sides with a semi-permeable membrane. The main aim of this study was to compare the efficiency of the AM under field conditions to the Pt uptake by native organisms, and to comment on the appropriateness of the AM as an alternative to living organisms as indicators of metal exposure. Previous studies have shown that the AM accumulates Pt in a concentration dependant manner. To validate the AM in the field, the Pt accumulation of the AM was assessed together with that of freshwater clams (*Corbicula fluminalis africana*), muscle and liver tissue of three fish species; sharp-toothed catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*) and Mozambique tilapia (*Oreochromis mossambicus*), as well as water hyacinths (*Eichhornia crassipes*) at two sampling sites. The two impoundments studied are located in a Pt mining area in South Africa. In Olifantsnek Dam the Pt is from geogenic sources, while Bospoort Dam is located downstream from several anthropogenic activities such as intensive Pt mining and refining activities, as well as urban and industrial effluents. Results from the field exposures demonstrated that the AM is a promising tool for monitoring Pt in the freshwater environment. The AM only represents the dissolved bioavailable metal fraction and does not consider, e.g. particle bound metals, which can be taken up by living organisms via the food. Thus, it is recommended to combine active monitoring using living bioindicators together with AMs to obtain insight into the role of dissolved bioavailable metal fractions and particulate bound metals in metal exposure in aquatic ecosystems.

3.19.P-We146 Rare Earth Elements in Soil Samples Derived From Electronic Waste: Bioavailability in Soil

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The dumping of waste electrical and electronic equipment (WEEE) and its recycling commonly known as “urban mining” has caused undesirable impacts on the environment. Many of these products are rich with rare earth and hazardous elements that end up in rubbish dumps and recycling centres. Harmful metals are exposed and leached into the environment. The fate of rare earth elements (REEs) through the disposal of electronic waste and urban mining into the environment has caused pollution and pose a threat to human health and the aquatic ecosystem. In this work, we report the results of several microcosm studies using conditioned soil to investigate the leaching of REEs in lab-scale experiments. The effective leaching of specific rare earth elements (La, Nd and Dy) were conducted under different experimental conditions. The effects of major variables on REE leaching were evaluated, which included lixiviant type and concentration, time, stirring speed, pH and solid to liquid ratio. The temperature was kept constant during all experiments at 23 ± 2 °C. The leaching efficiency of La, Nd and Dy was found to be significantly dependent on acid concentration and leaching time. The best leaching efficiency was obtained with 1 M HCl with a leaching time of 30 minutes, 300 rpm stirring, 50 g/L solid to liquid ratio. It was found that the leaching efficiency of 69%, 75% and 77% was achieved for La, Dy and Nd, respectively. In speciation studies, the results showed that 90% of REEs were obtained from acid-soluble fraction and residual fraction. The study is currently investigating the availability of the fractions, chemical behaviour in soil under environmental conditions, and the uptake of the REEs by biota.

3.19.P-We147 First Assessment of Rare Earth Elements Bioaccumulation in the Common Sole (*Solea solea*) Along a Continuum Between the Gironde Estuary and Its West Mud Patch (France)

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Through their sedimentary and biogeochemical characteristics, the River-dominated Ocean MARGin (RIOMAR) systems constitute key components for the ecosystem functioning of coastal areas and are especially important for the delivery of ecosystem services to mankind. The West Gironde Mud Patch (WGMP) is a sedimentary body about 420 km² large in the Bay of Biscay supplied by the Gironde Estuary inputs. The WGMP is a key area for early life stages of many exploited marine species, especially the common sole (*Solea solea*), an emblematic species highly dependent on this habitat. These marine species representing a strong economic interest for fisheries are increasingly exposed to emerging metallic contaminants, such as the Rare Earth Elements (REE). Growing consumption of REE has led to increasing transfer of these contaminants in the Gironde Estuary.

There is thus a real need for monitoring REE contamination in the common sole of the WGMP. This study aims to understand the spatial and temporal contamination patterns (sampling location and seasons) as well as the influence of intraspecific variations (length, weight, sex) of REE contamination in the common sole by comparing contamination between coastal and estuarine systems. The first data on REE concentrations in tissues of this species collected during two contrasting seasons at different locations in the WGMP (April and November 2021; MAGMA cruises, French Oceanographic Fleet) and in the Gironde Estuary (April 2015, April 2016 and October 2016; MOMBASAR cruises) are presented. Although data from coastal samples are still in process, our first results obtained for estuarine common sole showed the mean value of the sum of REE concentrations had a large variation range for small size/weight ($571 \pm 489 \mu\text{g}/\text{kg d.w.}$, $n=11$) corresponding to early life stages, whereas large fish had 3-fold lower values ($176 \pm 127 \mu\text{g}/\text{kg d.w.}$, $n=7$), probably attributed to weight dilution. Interestingly, three metals represent the majority of REE with Ce (40%), Nd (21%) and La (20%). This abundance corresponds to sea water composition and was previously observed in yellow eels in another French system (Loire Estuary). This study may greatly contribute to the future understanding of biogeochemical processes as a prerequisite for ecotoxicological risk assessment of emerging metals and subsequent regulations on metal exposure of marine species in the coastal domain.

3.19.P-We148 Spatiotemporal Biomonitoring of Rare Earth Elements and Yttrium Along the Norwegian Coast Using Cultivated Sugar Kelp *Saccharina latissima*

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The growing human population has led to an increased demand and use of raw materials including rare earth elements (the 15 lanthanoids and yttrium, REY). As technology critical elements, REY are used in a wide range of applications, including energy technology, electronics and lighting systems, metallurgical and automotive industries, and medical applications. In this study, we assessed the spatial and temporal patterns of REY in the sugar kelp *Saccharina latissima*, a key organism at ecological, economical and societal level. REY accumulation patterns were evaluated in relation to different local abiotic and biotic conditions and potential anthropogenic sources were identified. Cultivated *S. latissima* specimens were collected from 2 depths (1-2 m and 8-9 m) in 4 locations at 4 time points (from May to August) along the Norwegian coast over a germinating growth season REY concentrations were determined using ICP-MS in freeze dried samples ($n=96$), each comprising a pool of 10 specimens.

Relationships between element concentrations, algae growth (thallus length and frond width) and environmental parameters were evaluated. The results showed a high location-dependent variability in element concentrations and patterns. Specimens that were cultivated far from the freshwater riverine inputs exhibited higher growth over time, accompanied by increasing REY concentrations in function of their thallus length and frond width. Conversely, seaweeds from locations potentially affected by high fresh-water inputs reached their maximum growth earlier and exhibited different REY concentrations at different timepoints, irrespective of the dimensional growth trends. Cultivation depth did not appear to play a major role in REY concentration patterns. The results from this study provide information about spatial and temporal distribution of REY in marine environment along the Norwegian coast and elucidate the capacity of seaweeds to accumulate and sequester these potentially toxic elements from the environment.

3.19.P-We149 Sources and Fate of Metals and Metalloids in Wastewater Treatment PLANTS

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Sewage Treatment Plants (STPs) have been identified as significant sources of trace element emissions to aquatic environments. There is a need to gain a better understanding of the magnitude of the many sources of trace elements to STPs in order to be able to manage them in effluents if required. This study developed methods and applied them to derive an understanding of the proportions of inputs of aluminium, arsenic, cadmium, copper, nickel, molybdenum, silver, and zinc into wastewaters which are attributable to different urban sources. Furthermore, we have assessed the importance of ambient background levels of these metals/metalloids relative to anthropogenic sources in wastewaters as well as assessing the fate of metals during the wastewater treatment process. Of particular importance is to identify gaps in information and areas of uncertainty. Sources were split into domestic (mains tap water, faeces, urine and other sources including plumbing and activity related discharges), urban runoff (brake, tyre and road abrasion, exhaust emissions, oil loss and atmospheric deposition), service industry discharges, industrial emissions. Key specific sources included copper from brake lining abrasion and zinc in vehicle tyre abrasion. Significant variations were observed between countries depending on, for example, patterns of use of copper in plumbing and architecture and metals from industrial discharges tending to be more significant in eastern Europe. For many of the other elements the distribution of emissions between the sources was more evenly distributed reflecting their ubiquitous presence within the urban environment. Runoff sources were typically influenced by traffic density and degree of urbanisation. Background inputs were in most cases less than 25% of the overall contribution reflecting the current and historical use of these elements in urban environments. The removal of the elements from water within the sewage treatment process is generally efficient: cadmium, copper, zinc, aluminium and silver exhibit typically more than 70% removal to sludge for secondary treatment, with nickel and molybdenum over 50%. Only arsenic exhibited poor removal (< 10%) which may reflect a lack of data and/or the fact that it is the only element assessed that is an anion which may result in less sorption to particulates that themselves are often negatively charged. Significant uncertainty exists for many urban sources of aluminium, molybdenum, silver, and arsenic.

3.19.P-We150 The Impact of pH and Material Choice on the Toxicity of Elements

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Elements such as copper, zinc, cobalt and lithium can be essential for metabolic processes of many different organisms but are toxic in high concentrations. An increasing amount of modern industrial processes heavily influence the release of such elements to the environment. Mining and wastewater discharge are prominent examples of anthropogenic discharges into aquatic environments. Physico-chemical properties of the water, like temperature, pH, hardness, and conductivity control the speciation of such elements. This in turn influences their solubility, bioavailability, and consequently their toxicity. The toxic levels and effects of some elements like copper and lead to aquatic systems are quite well characterised. For other elements, such as lithium, this information is still scarce. To assess the toxicity of different elements, the zebrafish embryo toxicity test is a commonly used model. It is a very well described and widely used test systems in ecotoxicology. In addition to physico-chemical parameters, the material of the test vessel used influences element availability by affecting their adsorption to the vessel's surface. However, the interactions between the element tested and the material used to perform the fish embryo toxicity tests in have rarely been considered in the past. With this project, we want to fill some of these knowledge gaps by investigating, whether: (i) a decreasing pH impacts the toxicity of different elements and (ii) if the material used for the tests have an influence on metal availability and therefore toxicity. To address these aims, the zebrafish embryo tests using lithium, zinc, copper, and cobalt will be performed in standardised water. Afterwards, 120 hours after fertilisation, larval behaviour will be investigated. The test will be performed at pH 6 and at an unmodified pH 7.6. We will quantify the elements in the test solutions before and after performing the tests. Moreover, we will quantify their bioaccumulation in the larvae. With this data we aim at improving our understanding of element availability, their uptake, and toxicity under different test conditions and the importance of test vessel material choice.

3.19.P-We151 Accumulation and Effect of Cobalt in River Biofilms

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Cobalt (Co) is an essential trace element involved in several biomolecules. Over the last decade, industrial demand has led to an exponential increase of its extraction. Indeed, Co is intensively used in the energy storage technologies, such as in Co-lithium batteries found in electrical appliances and cars. An increase in dissolved Co concentrations is thus observed in aquatic environments, which might lead to negative impacts on living organisms such as river biofilms. These biofilms, at the base of the trophic network, are composed of bacteria, microalgae, fungi and microfauna. However, little is still known about Co accumulation and toxicity towards biofilms, which prevents adequate protection of freshwater environments. The objective of this study is to better understand the short- and long-term effects of Co on microalgal communities. To this end, mature biofilms were exposed to increasing concentrations of Co (0, 10^{-7} , 10^{-6} , $5 \cdot 10^{-6}$ and 10^{-5} M) for 7 days using outdoor microcosms (15 L) filled with the *Gave de Pau* water. Every day the exposure medium was sampled to analyse the concentrations of metals, cations, anions and dissolved organic carbon. Biofilms were also collected after 1, 3 and 7 days of exposure and were examined for different biological parameters. Firstly, the total and intracellular Co concentrations in biofilms were measured after an EDTA wash and acid digestion. This EDTA step allowed us distinguishing between total and intracellular content. The effect of Co will be evaluate at the subcellular levels using untargeted metabolomic analysis and the chlorophyll-a content while shift in microalgal community will be assessed using metabarcoding. In view of the first results obtained concerning the accumulation of Co by biofilms, bioaccumulated Co was mostly intracellular. Very good correlations between dissolved Co concentrations in the exposure medium and intracellular concentrations at each exposure time are observed (R^2_{adj} of 0.91, 0.95 and 0.97 respectively after 1, 3 and 7 days of exposure). The analysis of the various biological parameters could allow the characterisation of potential new biomarkers of exposure and the identification of bioindicator species within the exposed biofilms.

3.19.P-We152 Bioavailability and Colloidal Characterization of Heavy Metals and Radionuclides in Waterlogged, Contaminated Belgian Soils Via Diffusive Gradients in Thin Films

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The "Sigmaplan" is a Belgian programme that aims to permanently flood different lands of Flanders to favour the downstream resilience against the flooding effects of climate change. Some of these areas involve the Grote Nete and the Winterbeek rivers, contaminated by heavy metals and also by radioactive materials, both naturally occurring (NORM) and coming from anthropogenic sources as a result of historical discharges from the phosphate and nuclear industries. However, the impact of this new waterlogged (anaerobic) state on the bioavailability, colloidal behavior, and fate of these elements of concern still remains unknown. This research intends to determine ex situ the labile pools of Cd, Zn, Ni, As, Pb, Cs, Ra and U in the Grote Nete and Winterbeek soils, both in oxic and anoxic conditions. To do so, we will deploy Diffusive Gradient in Thin Films (DGT) devices in both soils, under different simulated reducing conditions. This technique has recently seen large developments for concentration analysis of different elements in pore waters. We will consider DGTs piston-shaped devices containing two different commercially available binding gels (titanium oxide and styrene divinylbenzene) and one self-made gel (zirconium oxide). These binding gels will be further analysed through laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and gamma spectrometry. We will place special attention to the challenging, low radionuclide concentrations of these soils, as well as the redox sensitive elements. We will present the results and discussion arising from this study and its influence in each element's solid-liquid distribution coefficient (K_D), which we intend to define as a radiological showcase for mixed contaminants.

3.19.P-We153 Evaluating the Protectiveness of the EQS for Zinc

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Zinc is both naturally occurring and widely used within society for a variety of different purposes. The combination of these factors means that zinc is ubiquitous in surface waters and Environmental Quality Standards (EQS) have been established to regulate zinc levels in the freshwater environment in many countries. This study has evaluated the sensitivity of benthic invertebrate communities in England to zinc exposures in order to evaluate whether they are adequately protected by the EQS for zinc. The analyses considered the abundance of specific species, genera, or families of benthic macroinvertebrates in relation to bioavailable zinc exposures. The analyses focused on the most commonly recorded taxa at sites for which sufficient chemistry information was available to calculate bioavailable zinc concentrations, to maximise the amount of data that could be included in the analysis. Eight species were identified as being potentially responsive to zinc exposures, and another eight species were identified as being unresponsive to zinc exposures. Thresholds for a reduction in the abundance of several groups of zinc sensitive taxa were derived and indicate that the EQS for zinc is sufficiently protective of the abundance of these sensitive species.

3.19.P-We154 Seasonal and Spatial WATER Quality Variations in the Karst Riverine Ecosystem

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Karst rivers are characterized by complex and unpredictable variations in water quality due to the strong interaction between surface and groundwater, as well as high sensitivity to pollution. The present study aimed to evaluate the seasonal and spatial variations in physicochemical, organic (COD, TOC, DOC, mineral oils) and inorganic (total and dissolved Al, As, Ba, Ca, Cd, Co, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Rb, Sb, Sr, U, V, Zn) parameters in the karst Krka River, Croatia. Although most of the Krka River watercourse is protected as a national park (NP), it is affected by improperly treated upstream wastewater discharges. The sampling was performed in four seasons of 2021 at eight sites: Krka River source (KRS, reference site), industrial wastewater from the screw factory (IWW), municipal sewage (MWW), tributaries Krpa (TKR, directly influences KRS), Orašnica (TOR, impacted by IWW), Kosovica (TKO, situated nearby gypsum factory), Butišnica (TBU, influenced by agricultural runoff) and Brljan Lake (KBL, located in the Krka NP). Water quality was more site than season dependent and, in all seasons, showed poor ecological status for IWW and moderate for MWW and TOR according to Directive on water quality status, especially for nutrients, TOC, DOC, and mineral oils. Compared to KRS, poorer values of some physicochemical water parameters were also recorded at TBU and TKO, as a result of agricultural and industrial activity, respectively. Dissolved and particulate metal(loid) forms showed comparable variations, with the highest levels at IWW, especially those used in industry (Sr, Fe, Zn, Mn, Ba, Al), but increase of many elements was also evident at MWW and TOR compared to KRS in all seasons. Seasonal variations of physicochemical water parameters were not obvious, especially at wastewater impacted sites which reflected discharge peaks. Concentrations of TOC, DOC, and mineral oils were higher during the wet season when transport from the catchment area and exchange between surface and groundwater were increased by the rainfall. However, phenol and metal(loid) levels were higher during the dry period, due to lower water levels and less effective purification processes in the river. Presented results pointed to deteriorated water quality near wastewater outlets, which also affect natural seasonal variations of the main ecological parameters. Proper purification of the wastewaters and monitoring plans are mandatory to protect this sensitive karst ecosystem.

3.19.P-We155 Cellular Mechanisms Involved in Cr(III) Uptake and Excretion : A Review

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Chromium (Cr) exists in several oxidation states; the most common being hexavalent Cr (Cr(VI)) and trivalent Cr (Cr(III)). Various industrial activities (e.g. steel mills, tanneries) are associated with significant releases of Cr(III) or Cr(VI) into the environment. The toxicity of Cr(VI) is well established and the mechanisms involved in its cellular uptake and handling quite well documented. In particular, sulfate (SO₄²⁻) transporters import chromate (CrO₄²⁻) in prokaryotic and eukaryotic cells because of the structural homology between the two ions. Furthermore, in presence of CrO₄²⁻, bacteria and several ascomycetes express a plasma membrane protein involved in CrO₄²⁻ export from the cytoplasm to prevent Cr(VI) toxicity. The reduction of Cr(VI) is also associated with reactive oxygen species (ROS) production. The increase of ROS generates oxidative stress in cell and causes DNA and protein damage. At the opposite, Cr(III) is often considered as non-toxic despite increasing evidence to the contrary. The role of Cr(III) as an essential human micro-nutrient is also increasingly questioned. However, the understanding of the mechanisms of Cr(III) entry and action in cells remains fragmented. Several studies demonstrated a relationship between Cr(III) and Fe(III) metabolism and import. In yeast, a pretreatment with Fe(III) protected against Cr(III) toxicity, suggesting a competition between Cr(III) and Fe(III) transport in cells. This hypothesis is supported by observations in in vivo models where treatment with Cr(III) limited Fe(III) absorption. Cr(III) is also known to bind transferrin, the major iron transport protein in the blood. Finally, at physiological pH, Cr(III) precipitates and forms insoluble particles of Cr₂O₃ that can be taken up in cells by phagocytosis or endocytosis. The internalized Cr(III) may then be solubilized in lysosomes by acidification and released in cytoplasm. In this contribution, we review the mechanisms involved in Cr(III) uptake and intracellular release in various organisms. This information will provide valuable hints for an improved understanding of the toxicity mechanisms associated with the two most common oxidation states of chromium.

3.19.P-We156 Ecotoxicity of Cr (III) and Cr (VI) Mixture to Freshwater Algae

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In the environment, Chromium (Cr) has two main redox states: Cr(VI) and Cr(III). Despite the consensus that Cr(VI) is more toxic than Cr(III), an increasing number of studies shows that both oxidation states are potentially toxic to organisms. In addition to this, Cr(VI) and Cr(III) often occur simultaneously in natural environments so that biota can be exposed to a mixture of the two forms. However, the ecotoxicity of Cr(VI) + Cr(III) mixtures is practically unknown. The present study evaluates the ecotoxicity of Cr (III)/Cr (VI) mixtures toward the model freshwater green algal *Raphidocelis subcapitata*. Algal tests were carried for individual Cr oxidation states and for their mixtures following the ISO standard 8692. Average EC₅₀ values were 0.099 ± 0.009 for Cr(VI) (n=40) and 0.059 ± 0.04 for Cr(III) (n=7). Mixture ecotoxicity was tested using binary combinations of the EC₅₀ and EC₁₀ of the two oxidation states. The corresponding experimental results were best described by a response addition model, suggesting different modes of actions for the two forms and no interactions between them. These observations agree with the known differences in the mode of action of Cr(III) and Cr(VI). Future studies should further examine the ecotoxicity of Cr(III) and Cr(VI) mixtures with real-world relative abundances in addition to the laboratory-based EC_x used for the present contribution.

3.19.P-We157 Cadmium Uptake and Its Effect on the Composition of Mineral Nutrients in Brassica Napus Grown Under Current and Future Climate Conditions

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Increasing soil contamination by heavy metals is a global environmental concern as they endanger ecosystems and pose a threat to human health when entering the food chain. Of these toxic metals, cadmium (Cd) does not play any beneficial role in plants, while its toxicity in plants depends on the bioavailability of Cd in soils and the concentration of elements that can compete with Cd during plant uptake. Through competition with nutrients, Cd has a significant effect on their uptake and distribution in the plant. However, Cd-induced responses on the uptake of plant nutrients are not straightforward. The control of Cd accumulation in plants is complicated by the fact that most of the essential nutrient transporters could also facilitate Cd uptake. In this way, similarities in physicochemical properties between Cd and other cations may interfere with the uptake of the latter. The bioavailability of Cd in soil, and therefore its phytoextraction, is influenced by many environmental factors, including climate change factors, the most important of which are the increasing concentration of carbon dioxide (CO₂) in the atmosphere and temperature. Yet knowledge in regard to how these climate change-related factors interact with Cd exposure and downstream responses seems to be lacking. Therefore, the aim of this study is to elucidate if Cd affects plant uptake of essential elements from the soil in differential ways under current and future warmer climate conditions. For this purpose, we conducted the pot experiment with the rapeseed (*Brassica napus* L.) as the model plant in climate chambers under current climate (21/14 °C and 400 μmol mol⁻¹ CO₂) and future climate (25/18 °C and 800 μmol mol⁻¹ CO₂) conditions. Rapeseed is not only an important oilseed crop worldwide, but also an emerging biofuel crop and, like other *Brassica* species, has many features, like high biomass production, rapid growth rate, and ability to sequester Cd, suitable for the phytomanagement of Cd-contaminated soils. The content of Cd and mineral nutrients along with the interactions between Cd and nutrients in rapeseed were investigated. Acknowledgments: This research is funded by the European Social Fund under the No 09.3.3-LMT-K-712 “Development of Competences of Scientists, other Researchers and Students through Practical Research Activities” measure.

3.19.P-We158 An Integrated Analytical Approach Reveals the Distribution of Cadmium Uptake by Potato (*Solanum tuberosum*) Roots and the Effects on Root Viability and Plant Development

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Potato (*Solanum tuberosum* L.) is the fourth most important crop in Europe and the third most important in the world, behind only rice and wheat. It is one of the main foods for humankind, consumed by more than one billion people worldwide due to its composition, gastronomic and technological versatility, as well as the low market price of tubers. Roots of potato plants develop protective tissue layers, endodermis and exodermis, which accumulate lignin (a phenolic polymer) and suberin (a fatty-acyl derived polymer) to regulate the uptake of nutrients and toxic elements [1]. Cadmium is toxic to plant cells, even at low concentrations. The roots of plants generally avoid Cd-enriched patches in soils. Shoot Cd concentrations are determined largely by Cd uptake by root, sequestration within root vacuoles, translocation into the xylem and phloem, transportation to other organs and dilution within the shoot through growth [2]. It has been described that potato is strongly affected by Cd toxicity, producing a reduction in biomass accumulation [3]. We have studied the effect of Cd in potato plants grown in hydroponic solution enriched with Cd (from 0 to 200 μM) at different exposure times. We have evaluated the effects on the shoot and root growth and their correlation with cell toxicity through vital staining of roots with propidium iodide/fluorescein diacetate, and lipid peroxidation analysis. As for metal accumulation, Cd in roots and shoots have been measured with ICP-MS after acid digestion. Other elements such as Ca, Fe, K, Mg, Cu, Mn, P, Zn, and Mo, have been included in the analysis to investigate in which extend the Cd uptake affects elemental concentration and translocation. Finally, micro-PIXE (proton induced X-ray emission) has been used to emphasize the differential distribution of metals in root sections [4]. **References** [1] Company-Arumí et al. *Phytochem Anal* 27 (2016) 326-335. [2] Lux et al. *J Exp Bot*, 62 (1) (2011) 21-37. [3] Dorneles et al. *Adv Hort Sci* 33(1) (2019) 46-56. [4] Cestone et al. *Sci. Total Environ.* 427-428 (2012) 339-346.

3.19.P-We159 Within Body Distribution of Metal Contaminants in Brown Trout (*Salmo trutta Fario*) and the Potential of Fish Blood As a Non-Lethal Monitoring Tool

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The use of fish as a monitoring tool of potential metal contamination in the surrounding environment is well established, and most often focus on internal tissues such as the liver and/or muscle tissues. Although successful, there are, however, clear disadvantages to this method. For example, not only does it require the sacrifice of a large number of fish but since it also requires dissection of each individual to extract the targeted tissues it can be rather time consuming. As such, there is a need to develop reliable and non-lethal methods for monitoring metal contamination loads in fish and other aquatic biota. Drawing on iCap TQ-ICP-MS-data, we explore blood as a potential (non-lethal) method to measure trace metal concentrations in brown trout (*Salmo trutta fario*) using small volumes of blood (< 0.5 mL). We also assess potential differences in contamination loads in different components of the blood (i.e. whole blood vs plasma vs red blood cells) and how these relate to each other. Finally, we also decipher the within individual distribution (i.e. blood, liver, kidney, bile, muscle and gonads) of metal contaminants (e.g. Pb, Cd, and Hg) and assess how these contamination loads relates to that of the surrounding environment (soil, sediment and biofilm). We will show that, based on initial results, even small quantities of fish blood appear to be a promising and reliable tool to monitor metal contamination in aquatic environments.

3.19.P-We160 Small Players in the Big League: Fish Parasites As Effect Indicators of Metal(Loid)S in the Marine Environment

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Environmental parasitology has been used as an ecotoxicological assessment tool to determine the impact of anthropogenic activities on parasites, relating deviations to environmental health. Certain fish parasite taxa are known to accumulate metal(loid)s and to reduce the levels of toxic substances in infected hosts, thereby balancing the uptake and elimination rates more readily. In the present study, parasite taxa are considered as effect indicators, focussing on the effect of pollutants on changes in the parasite community structure of the marine fish host, *Clinus superciliosus* (Super klipfish) from various localities along the South African coast. The metazoan parasites of the selected host were sampled from four localities (two commercial harbours, west coast; and two relatively pristine localities, southeast coast). The parasite community structures of 55 klipfish were examined, and muscle and liver tissue were analysed to determine metal(loid) concentrations (As, Cd, Cu, Fe, Mn, Pb, Sn, Sb, Zn). Results from linear regression models comparing infection intensity with metal concentrations, indicate a decrease in metal(loid) concentrations with an increase in parasite load. From the redundancy analysis and general linear mixed models, helminths of the orders Acanthocephala and Cestoda associated with concentrations of As, Cu, Mn, Pb and Sn, as well as As, Cd, Cu, Fe, Mn and Zn, respectively, specifically at the two southeast coast sites. The results from the present study are supported by previous findings where concentrations of metal(loid)s measured in acanthocephalans and cestodes accumulated in a higher magnitude than its associated hosts. This is an important interdisciplinary approach, applicable to a wide range of systems but scarcely used on marine ecosystems, which can contribute to environmental monitoring programs.

3.19.P-We161 Assessment of Metal Bioconcentration in a Transboundary River: The Lower Olifants (South Africa) and Massingir Dam (Mozambique)

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The Massingir Dam was built on the Olifants River, which is regarded as one of the most mineralized and polluted rivers in South Africa and has received considerable attention due to increased anthropogenic activities. Although the Olifants River is well studied in South Africa, there are limited studies on the exposure and effect of pollutants such as metals in the lower reaches of the river and no published studies at all on the ecotoxicological status of the Olifants River in Mozambique. The present study intends to determine the concentrations and distribution of selected metal on a spatial and temporal scale utilizing selected fishes from lower Olifants at Kruger National Park (KNP) and Massingir Dam. For this purpose, water, sediment and fish (*Oreochromis mossambicus*, *Clarias gariepinus* and *Hydrocynus vittatus*) samples were collected from three sites in the KNP (Mamba weir, Balule and Gorges) and Massingir Dam during low-flow periods of 2019 to 2020 for metals content analysis. The analysis of selected metals in different matrices of the two-sampling site revealed spatial and temporal variations and the first survey with highest bioconcentration, although the samples were collected at the same period, i.e., low flow. Zinc was the metal with highest concentration in water and fish and in Massingir Dam than KNP. For the sediment, Cr was the metal with highest concentrations. There was a clear bioaccumulative trend for Hg since it was lower in water followed by sediment, then fishes. The opposite was found for As, which was higher in water, sediment and finally fishes. All metals with the exception of Cr were higher in fishes from Massingir Dam. The sharptooth catfish, *C. gariepinus*, bioaccumulated more metals than the predatory tigerfish, *H. vittatus*, indicating that sediments are probably the main source of metals. Cadmium was the metal with lowest concentration for all samples in both surveys and sites. This study brings new, updated and important data on the metal pollution status of Olifants

River, indicating more surveys are needed in the transboundary regions to allow a better understanding of the pollution level on this system.

3.19.P-We162 Transfer of Lead From Ancient Shot Pellets to Sediments in a Mediterranean Floodplain

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The highest densities of lead shot pellets in sediment from Europe have been detected in waterfowl wintering areas in Mediterranean wetlands. Continued waterfowl hunting from the same hunting blinds originates high densities of shot pellets in sediment in the surrounding 250 m. Wetlands in Denmark, France and Spain show average densities of more than 100 pellets/m² in the top 20 cm of sediment. The persistence of pellets in sediment has been estimated around 30–300 years with the consequent risk of Pb poisoning, a significant cause of mortality for many waterbirds species. Lead pellets at the bottom of wetlands contaminate water and sediment due to mechanical corrosion and chemical reactions under specific conditions. In addition, contamination by other trace elements, such as mercury, antimony and arsenic, can be also associated with hunting in the past. Therefore, research that verifies levels of trace element pollution in wetlands where hunting has been practiced in the past is crucial. This study was carried out in Tablas de Daimiel National Park, a protected floodplain wetland from the Iberian Peninsula that suffered heavy hunting pressure during the late 19th and early 20th century. To determine the density of lead shot pellets (shot pellets/m²), sediment samples were taken along eight transects following the cardinal points around a historic hunting blind (Puesto del Rey). To evaluate the sediment contamination by lead and other elements, samples were collected randomly in NW and SE transects and in the vicinity of the hunting blind. Lead pellets in sediments were isolated by wet-sieving and sediment samples were acid-digested and Pb, As, Hg, Se, Sb concentration was measured by ICP-MS. Pellets in sediment ranged between total absence to a maximum of 707 pellets/m², with the highest density in the NW transect. Accordingly, sediment Pb concentrations in the NW transect were higher than in the SE transect and increased with the depth and number of pellets. Hg concentrations did not show significant differences between areas, but a slightly higher value was found close to the hunting blind. These results suggest that shot pellets can transfer lead to the surrounding sediment, creating a problem of metal pollution in wetlands that have suffered or are currently suffering a high hunting pressure.

3.19.P-We163 Photocatalytic Degradation of Rhodamine B Dye by Molybdenum-Doped ZnO Nanoparticles and Its Consequences for Danio rerio

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The degradation of water pollutants with different photocatalysts are studied extensively in the last years, but potential impacts caused by degradation byproducts are still largely ignored. In this study Molybdenum doped Zinc oxide nanoparticles (Mo-ZnO NPs) were synthesized with the hydrothermal method and the NPs photocatalytic performance was assessed using the degradation of Rhodamine B (RhB) as proxy. Acute and sub-lethal toxicity of NPs, RhB and its (by)product were tested using *Danio rerio*. Fish were exposed for up to 4 and 15 days following OECD 203 and 204, respectively. Acute tests were done in triplicate with 21 fish in each group (i.e., with seven specimens per replicate) to determine the 96-hour median lethal concentration (LC50) of Mo-ZnO NPs, Rhodamine B and the photocatalytic degradation (by)product. The sub-lethal toxicity assessment was run with two replicates per treatment containing 10 specimen per replicate. The second test employed in total four treatments a control, a sublethal concentration of Mo-ZnO (100mg/l) or RhB (10mg/l) as well as the photocatalytic degradation (by)product diluted by 50 %. As endpoints changes in oxidative stress biomarkers such as total oxidant species (TOS), catalase activity (CAT), superoxide dismutase (SOD), glutathione content (GSH) malondialdehyde (MDA) in the liver, brain and ovary tissues were measured. The photocatalytic experiments indicated a removal efficiency of 95 % of the dye when applied at 5 mg/l together with a NPs concentration of 100 mg/l over 30 minutes under visible light. Acute tests indicated the 96 h LC50 of 482.71 + 0.56 mg/l and 23.176 + 0.147 mg/l for Mo-ZnO and RhB, respectively. For photocatalytic degradation (by)product no EC50 was calculated due to low mortality (only 1 at the highest concentration). In comparison to control group, mean CAT and GSH activity decreased but MDA which is known as a marker of oxidative stress and lipid peroxidation increased in all treatments. TOS was raised in presence of RhB and of photocatalytic degradation (by)products compared to control group while it decreased in the Mo-ZnO treatment. SOD activity was enhanced in Mo-ZnO and byproduct treatments but declined in RhB treatment. The results suggest that despite high removal efficiency of RhB by Mo-ZnO NPs, changes in oxidative stress markers induced by the (by)products did not significantly decrease compared to initial pollutant, accordingly further studies are needed for a better understanding of its ecological consequences.

3.19.P-We164 Microbiota-Mediated Transformations of Arsenic Species From Rice in Mammals

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According to the World Health Organization, arsenic is one of the ten pollutants of major health concern, with more than 200 million people worldwide being at risk of arsenic exposure from their diet. In particular, rice accumulates up to 0.4 µg/g of arsenic, of which 85 – 90 % corresponds arsenous acid (AsIII) and arsenic acid (AsV); and the remaining to methylarsonic acid (MMAsV) and dimethylarsinic acid (DMAsV). Although all four arsenic species are classified as carcinogens by the IARC, their

specific modes of toxic action are strongly related to their metabolites once in the human body. In addition, interpopulation variability can influence arsenic metabolic products and excretion rates, which depend on human genetics and gut microbiome. Understanding the effect of gut microbial communities on arsenic biotransformations can help to prevent the adverse effects of chronic arsenic exposure in humans. This study aims to unveil the microbial-mediated transformations of AsIII, AsV, MMAsV, DMAsV from rice in the mammalian gut. Specific pathogen free (SPF) and germ free (GF) mice were fed rice-containing chow diets at varying concentrations of inorganic and organic arsenic species. After seven weeks of chronic arsenic exposure, mice were euthanized and all gut contents and key organs involved in arsenic metabolism were analysed for arsenic speciation using high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS). Our findings show how the gut microbiome plays an important role in the methylation of arsenic from diet but also promotes the transformation of arsenic into the more toxic thiolated arsenic species. In addition, we identified differences on the specific arsenic excretion pathways in the absence and presence of gut microbiota. The results from this study provide valuable insights to effectively understand how the mammalian gut can help in mitigating the pernicious effects of dietary exposure to arsenic.

3.19.P-We165 Methylmercury Bioaccumulation in Coastal Invertebrates in the Minas Basin, Bay of Fundy

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Methylmercury (MeHg) is a neurotoxin produced in estuarine sediments that bioaccumulates and biomagnifies in ecosystems and has negative impacts on organism health. Studies on MeHg bioaccumulation in coastal invertebrates at the base of the food web in Eastern Canada are limited, and this data is necessary to determine risk for bioaccumulation in higher trophic level organisms like fish and migratory birds. Specifically, the Minas Basin in the Upper Bay of Fundy provides critical habitat for fish spawning, and is an important stopover site for over 30 species of shorebirds during their southern migration. Fourteen families of invertebrates were collected from 5 sites in the Minas Basin during summer 2021, and analysed for concentrations of MeHg and THg, and stable isotopes of ^{15}N and ^{13}C . Sites varied significantly in sediment THg (ANOVA $p < 0.001$), with mean concentrations ranging from 2.01 ng/g to 25.56 ng/g (dw). No clear relationship was seen between sediment THg and invertebrate THg or MeHg. Most invertebrate families had concentrations of MeHg below the Canadian tissue residue guideline for the protection of wildlife consumers of aquatic biota of 33 ng/g, but polychaete families (Goniadidae, Maldanidae, and Phyllodocidae) and mud snails (Nassaridae) had some individuals with MeHg levels above this guideline. Relative trophic position of invertebrates was determined using ^{15}N , and mean ^{15}N for families ranged from 6.72 (Corophiidae) to 14.14 (Goniadidae). A positive correlation was seen between MeHg levels and ^{15}N in invertebrate families, consistent with the bioaccumulative nature of MeHg in the food web.

3.20 Using measured concentrations of chemicals in environmental risk assessment: Improving the understanding of uncertainties (Poster)

3.20.P-We166 Evaluation of Component-Based Risk Metrics and Their Impact on Mixture Assessment Using 56 European WWTP Effluents As a CASE Study

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Chemical prioritization and mixture considerations are key elements of environmental risk assessment as a basis for effective management. While several different assessment approaches are frequently used for this purpose, systematic comparison and guidance for usage are currently lacking. Screening 56 European WWTP effluents for 499 typical water contaminants with LC-HRMS, we detected 366 pharmaceuticals, biocides, pesticides and industrial chemicals and applied three different risk metrics including the regulatory MEC/PNEC approach, an aquatic-community directed approach based on species sensitivity distributions and multi-substance potentially affected fractions (msPAF) of species and a Toxic Unit (TU) approach addressing algae, invertebrates and fish as major Biological Quality Elements. Concentrations of the detected target compounds range from less than 1 ng/L up to more than 10^5 ng/L. In agreement with regulatory requirements and precautionary principle, the MEC/PNEC provided most extensive exceedance of the threshold of concern at all sites varying from 58 to 13000fold with strong predominance of compounds with high assessment factors, thus prioritizing for uncertainty rather than for known toxicity. Although in our case none of the effluents could be evaluated as safe, MEC/PNEC can be useful as a tier-1 approach identifying samples that do not require further consideration. The higher tier approaches based on TUs and msPAF resulted in typically lower exceedances discriminating safe from non-safe effluents. WWTP effluents after ozonation or activated carbon treatment were evaluated as safe. Both higher-tier approaches prioritized primarily data-rich chemicals of known toxicity including pesticides, biocides and some pharmaceuticals. While mixture assessment can be performed as a tiered approach, a consensus approach for compound prioritization is proposed suggesting 55 compounds of concern prioritized by at least two approaches.

3.20.P-We167 Worst-Case Prioritisation Ranking of Organic Chemicals Detected in Groundwater and Surface Waters in England

David Spurgeon, Centre for Ecology & Hydrology, United Kingdom

The UK Environment Agency has been using GC-MS and LC-MS scans to semi-quantitatively measure organic substances in groundwater and surface waters. Here we present an analysis to generate a worst-case ecological hazard ranking of the detected

substances from these groundwater and surface water sample analyses. Water Framework Directive EQSs, NORMAN Network PNECs and chronic species sensitivity distribution (SSD) HC50s were collected for all chemicals measured in the two monitoring programs. These hazard values were then compared to the highest measured concentration for each chemical separately for ranking. Hazard ranks were then multiplied by the substance detection frequency ranking to calculate a detection corrected average hazard rank for each chemical measured for each analytical method, i.e. GC-MS and LC-MS in groundwater and surface water. The separate rankings in groundwater and surface water allowed comparisons of the nature of the highest ranked chemicals in each media. Pesticides present in the top 30 highest ranked chemicals included legacy pesticides (particularly in groundwater) and current use actives (particularly in surface water). Intentional monitoring of specific uses were responsible for certain substances appearing in the top 30 ranked. A number of industrial and plastics associated chemicals were ranked highly in groundwater, while more consumer goods, personal care products and pharmaceuticals were ranked highly in surface waters. The ranked lists of substances from the comparison of monitoring and hazard data for groundwater and surface water identify a set of potential priority substances that are putatively of higher risk. This list can inform on the relative importance of sources. The final ranked lists can act as a prompt to more detailed regulatory assessments and future research.

3.20.P-We168 Environmental Risk Assessment of Organic Micropollutants in Public Wastewater Treatment Plants in Flanders, Belgium

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The widespread occurrence of organic micropollutants (OMPs) is a growing concern due to potential adverse effects on aquatic wildlife. WWTP effluent is a major source of OMPs that have been found in the form of complex chemical mixtures. The prioritization of concerned OMPs and WWTPs is a useful approach for water quality management, which can be done by using environmental risk assessment (ERA). In this study, the ERA was performed using the Flanders Environment Agency database of measured environmental concentration (MEC) of OMPs in 38 Belgian WWTP effluents from 2000-2020. The effect data were annual average environmental quality standards (AA-EQS) and chronic species sensitivity distributions (SSD) to derive risk quotients (RQ) and multi-substance potentially affected fraction of species (msPAF), respectively. The 27 OMPs from the database included 14 pharmaceuticals, 4 personal care products, 9 herbicides, and 3 others, which had MECs ranging from 2.35 ng/L to 360 µg/L. Predicted ecological risks were mainly driven by the anti-inflammation drug diclofenac, the herbicides flufenacet and diuron, and the personal care product 4-nonylphenol. The 28 WWTP effluents were at high risk of mixture effects with the sum of RQs exceeding 10 and msPAF between 5 to more than 25%. RQs and msPAF of the herbicides dominated among these WWTP effluents. Based on this observation, we wanted to validate the prediction of the environmental risks from the database with ecotoxicity testing of real-world wastewater treatment plant effluents. WWTP effluents with high predicted risk were selected to perform whole effluent toxicity testing with freshwater green algae (*Raphidocelis subcapitata*) and cyanobacteria (*Microcystis aeruginosa*), which are sensitive test organisms to herbicides. Our study highlights the importance of using ERA for the selection of candidate compounds and toxicity test systems to focus on future effect-based monitoring campaigns.

3.20.P-We169 Analysis of Public Environmental Monitoring Datasets for the Listing and Delisting of Priority Substances

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The procedure for analysing public environmental monitoring data to assess the listing and delisting of substances as priority substances (PS) under the associated directive (2455/2001/EC amended by 2013/39/EU) draws on the Watch List approach (Carvalho *et al.*, 2016). This compounds a series of worst-case assumptions resulting in an unrealistic risk assessment, notably, the manner in which it (i) deals with outliers and non-detections; (ii) considers all available data to assess the current state of the environment whilst ignoring changes in policy and associated changes in product registrations/usage and their impact on environmental residues; (iv) compares a single worst case predicted no-effect concentration (PNEC) against the 95th percentile measured environmental concentration when deriving the hazard quotient ignoring associated Water Framework Directive guidance and the updated annual average (AA) and maximum acceptable concentration (MAC) environmental quality standards (EQS). This poster uses a surrogate dataset for an example compound, compiled using readily available and more recent public monitoring datasets (given the dataset used in the draft PS dossier preparation is not available owing to Member State data access restrictions). We outline key issues relating to (i) data quality and harmonisation, (ii) data processing e.g. outlier definition and dealing with non-detects, (iii) sample number for confidence in site/country exceedance results and (iv) continuity of data for assessment of trends. The method of analysis explores the implications of using the proposed methodology, for example, using the substitution approach for dealing with non-detections which is acknowledged to introduce bias/error (Helsel, 2005; 2012) instead of the Kaplan Meier, Robust Regression on Ordered Statistics and/or Maximum Likelihood Estimation (MLE) approaches, depending on the rate of left-censored data and sample size. The analysis presented demonstrates how compounding these overly precautionary assumptions may lead to an assessment of risk that will require many MS to invest precious resources monitoring for a compound that does not present a risk to their surface waterbodies. More dialogue between regulatory agencies, industry and data analysis experts is required to define an approach that makes the most of the public monitoring data available but that also acknowledges the inherent challenges when doing so.

3.20.P-We170 Evaluating Exposure Data for Reliability and Relevance: Time to Balance the Efforts in Chemical Risk Assessment?

Graham Merrington¹ and **Lisa Nowell²**, (1)*WCA Environment Limited, United Kingdom*, (2)*U.S. Geological Survey, United States*

Environmental risk assessment is routinely performed through the comparison of measured concentrations of a chemical in environmental matrices or biota with some hazard metric, often based on effects of the respective chemical on organisms tested in laboratories. The ratio of the measured environmental concentrations, or exposure, to the ‘no effect’ or ‘effect’ concentration is termed the risk quotient (or risk characterisation ratio). Clearly this simple ratio has two input parameters, but historically a great deal more effort and energy is expended in compiling data on the ‘effects’ half of the ratio than evaluating exposures. Indeed, regulatory jurisdictions across the globe responsible for performing chemical risk assessments have established prescriptive frameworks and process to assist in the evaluation of ecotoxicity data reliability and relevance. Such evaluation ensures consistency of approach in the derivation of hazard metrics, such as Water Quality Guidelines or Criteria, Environmental Quality Standards (EQS), Aquatic-Life Benchmarks, and Predicted No Effect Concentrations (PNEC). In addition, these frameworks and processes, also provide those generating ecotoxicology data with clear pathways to follow to ensure such data is useful for regulatory purposes. This presentation will outline ‘best practice’ for the evaluation of chemical exposure data reliability and relevance. This work will also be useful to the authors of chemical exposure studies, as it will address information that needs to be provided to ensure that the resulting chemical dataset will be useful in chemical exposure assessment. This work is part of a SETAC Technical Workshop; Using Environmental Concentration Exposure Datasets in Environmental Assessments: The Development of Criteria for Reporting and Evaluating Exposure Datasets (CREED), from which the full outcomes will be published in the autumn of 2022.

3.20.P-We171 Criteria for Assessing the Relevance of Exposure Datasets

Adam Peters¹ and Claus Svendsen², (1)wca, United Kingdom, (2)CEH, Wallingford, United Kingdom

This poster provides a summary of the preliminary findings of the relevancy aspects of a SETAC workshop on the development of criteria for the reporting and evaluation of exposure datasets. The workshop has been supported by the Metals Environmental Research Associations (MERA), Cefic LRI, Syngenta, ERM (Environmental Risk Management), Unilever, GSK, BASF, BAYER, Arcadis, and Concawe. The relevance of any given dataset depends intrinsically upon the purpose to which it is intended to be applied. A consequence of this is that it is necessary to define the purpose of the analysis that is to be conducted on the dataset prior to making any assessment of whether or not it is relevant. In this aspect the assessment of the reliability and relevance of exposure datasets may be somewhat different to the assessment of the reliability and relevance of environmental hazard data, such as Klimisch or CRED, that is routinely used for the derivation of environmental effects thresholds such as Predicted No Effect Concentrations or Environmental Quality Standards. The reason for this difference is because the potential scope of application appears to be somewhat broader. The assessment of the relevance of a dataset is also more closely linked to the assessment of reliability than for comparable assessments of environmental hazard data. Relevance in relation to chemical exposure data addresses the suitability of the data for the intended purpose of use. The key questions are around how the purpose for which the dataset is being applied affect the type and diversity of data that it is appropriate. This may need to consider the sampling programme design, and the spatial and temporal frequency of samples, as well as the handling of censored data and how data are summarised.

Track 4: Ecological risk assessment and human health risk assessment of chemicals, mixtures and stressors and risk mitigation strategies

4.01 Advancing the field of nano- and microplastic toxicity exposure scenarios to better support risk assessments

4.01.T-01 Behavioral and Growth Effects of Natural and Synthetic Microfibers in Two Estuarine Indicator Species

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Microfibers are increasing in environmental samples and need closer look to identify which size, shape and type can affect organisms. To identify this, we used three types of microfibers in this study from rope samples used in boats and coastal systems. To compare natural with synthetic microfibers, we used organic cotton, polyester, and polypropylene microfibers (80-150 µm in size). Early life stages of estuarine indicator species Inland Silverside (*Menidia beryllina*) and mysid shrimp (*Americamysis bahia*) (1 day pre-hatch – 4 days post hatch; 7-14 days post hatch, respectively) were exposed to these three microfibers at three concentrations (3,10,30 particles/ml) in 5-25 PSU salinity range to mimic estuarine conditions and identify climate change impacts. The cotton microfibers were not detected in silversides and mysids shrimps when looked through Zeiss microscope. Whereas both the polyester and polypropylene microfibers were detected in stomach and gut line of the silversides. When compared with behavioral variability and growth effects, cotton demonstrated least behavioral impacts and no growth effects in both the organisms. In contrast, polyester and polypropylene were identified to have a significant effect on mysid and silverside behavior as well as growth in at least one salinity. To improve our understanding, we mix microfibers with tire particles and next step is to identify its impact on organisms. This raises concerns for ecosystem health and policies required to limit the microfiber outfall in aquatic environment.

4.01.T-02 The Effect of Shape and Polymer Type on the Toxicity of Microplastics in *Daphnia magna*

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The impact of microplastics on the environment has been extensively studied in the past decade. A common criticism is that the polyethylene (PE) and polystyrene (PS) microbeads used in these studies do not reflect the secondary microplastics (fibers or fragments, other polymers) commonly found in the environment. Hence, existing literature might underestimate the impact of microplastics on the environment. This study aims at providing a comparative assessment of the toxicity of microplastics in freshwater invertebrates by investigating the reproductive effects of the different polymers and shapes on *Daphnia magna*. Daphnids were exposed for 21 d to polyamide (PA) and polyethylene terephthalate (PET) fibers and fragments. PS beads and wool fibers were used as reference material for other studies and as a natural reference, respectively. Animals were exposed to four concentrations ranging from 2 to 250 mg/L with 10 replicates for each treatment and 20 negative controls. A deviation from the standard OECD test setup was the use of plankton wheels to keep the microplastics in suspension and maintain a consistent exposure concentration. The number of neonates produced during the experiment was recorded during every water exchange (thrice a week). The reproductive toxicity of microplastics in daphnids varied with polymer type and particle shape. This shows that the common approach of using a single type of microplastic cannot sufficiently predict the impacts of microplastics in general. Furthermore, the data show that the dose metric (e.g., mass, number or volume per liter) used to present the plastic concentration can have significant impact on the results and conclusion. Lastly, the concentration of PS beads in the test medium decreased over time, demonstrating the importance of determining the actual exposure concentrations throughout the experiment.

4.01.T-03 Ecological Risk Assessment of Microplastics in the San Francisco Bay Using a Bayesian Network Relative Risk Model

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There has been an increased interest in understanding and managing the impacts that microplastics may have on ecological systems, resulting in an increased need for a comprehensive risk assessment. The goal of this study was to conduct a spatially specific microplastic risk assessment for the San Francisco Bay. Additionally, we wanted to develop a framework that could be used for microplastic risk assessments in other regions. Microplastics are a complex, abundant contaminant and therefore require a risk assessment method that is able to consider the many variables that may impact overall risk. Using a Bayesian network relative risk model (BN-RRM), two models were produced based on likely differences in sources, transport, and toxicity: one model for tire wear particles and one model for other microplastics types. For this presentation, we will focus on the tire wear particle model. The model was parameterized with microplastic monitoring data from the San Francisco Estuary Institute and microplastic toxicity data from Oregon State University. These results can be used by regional stakeholder and decision makers to determine how concentrations of microplastics may affect important ecosystem endpoints. Desired outputs can also be selected, and the model will determine what the input must be to reach that goal. With the data that is currently available, a quantitative, spatially specific risk assessment is possible. Additionally, Bayesian networks are an excellent tool for modeling the complex and uncertain nature of microplastics in the environment. Bayesian networks can incorporate different types of data and easily be modified as new data becomes available, allowing for flexible and dynamic models to be created. Finally, we have noticed a significant lack of concentration-response data for microplastics and therefore this should be prioritized for microplastic toxicity testing.

4.01.T-04 First Steps Towards an Assessment of Plastic Risk to the Norwegian Environment (FARE)

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To understand the environmental effects of plastics it is necessary to set this within the context of risk. At its core, environmental risk is a function of the concentration of a pollutant in the environment (exposure level) and the effects that pollutant causes in organisms (hazard). To conduct a robust risk assessment of a particular pollutant it is necessary to have sufficient exposure and hazard data available. Recent studies have highlighted three critical limitations and knowledge gaps that are currently preventing risk assessment of plastic pollution in Norway and beyond, (i) an insufficient quantity of exposure and hazard data for macro- and microplastic [1], (ii) the variable quality of existing macro- and microplastic exposure and hazard data, and (iii) a lack of tools for assessing the quality. Regarding (ii) and (iii), the aim of this work was to develop and test a new toolbox specifically designed to assess the quality of existing macro- and microplastic exposure and effects data.

4.01.T-05 Bioaccumulation of Emerging Contaminants in Liver, Muscle and Plasma of Eel (*Anguilla anguilla*): Influence of Microplastics

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Emerging contaminants (ECs) are one of the most important indicators of the anthropic influence on the environment, and they have recently shown to interact with **microplastics (MPs)**, specially the pesticides, several PPCPs and a few PFASs contaminants. Several gaps remain on the accumulation and distribution of ECs in biota because only very partial data are available, as ecotoxicity studies rarely include tissue concentrations. Eels can be used as bioindicators of contamination, its **high protein and lipid content** (7-15% and 5-20% respectively in wet weight), pose a challenge for the extraction of organic pollutants, given their physiological and behavioral characteristics, which make them vulnerable and sensitive to the presence of emerging contaminants. The aim of this study is to assess the accumulation of a combination of ECs belonging to different chemical classes including pesticides, PFASs, PPCPs and MPs in visceral tissue of muscle and liver and plasma of eel (*Anguilla anguilla*). The validation of the method was carried out using **QuEChERS extraction** and a clean-up based on a new sorbent (**EMR-Lipid**) for the liver and muscle, and the solid phase extraction (**SPE**) for the plasma, and all of extracts were analyzed via **UHPLC-MS/MS**. For to evaluate the accumulation of ECs and the influence of MPs, eels were distributed into three groups:

control (B), exposed to ECs (C), and exposed to ECs and polyethylene MPs (C + M). The study was carried out during a 58 days period divided into two phases: (a) exposure phase (0-28 days), and (b) depuration phase (29-58 days). A total of 3 pesticides, 3 PFAS, and 2 PPCPs showed bioaccumulation in *eel muscle*, with bioaccumulation factors (BCFs), ranging between 1-342 L/kg, and 2 pesticides, 2 PFAS and 1PPCPs showed bioaccumulation in *liver samples*, with BCFs ranging between 1-295 L/Kg. Some ECs in plasma of *A. anguilla* have been determined, such as chlorpyrifos, chlorfenvinphos, etoricoxib, PFOA, PFOS, PFDA, PFBS, diclofenac, naproxen, triclosan e ibuprofen. The assessment of MPs influence in the bioaccumulation of organic contaminants requires deep knowledge about the sorption mechanisms of the contaminants to the different MPs in given environmental conditions. Acknowledgement This work has been supported by Grant RTI2018-097158-B-C31 funded by MCIN/AEI/ 10.13039/501100011033 and by “ERDF A way of making Europe”

4.01 Advancing the field of nano,- and microplastic toxicity exposure scenarios to better support risk assessments (Virtual Only)

4.01.V-01 Developmental and EpiGenetic Toxicity of NanoPlastics Using Embryotoxicity Platforms: Human STEM Cell and Zebrafish Embryo

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Nanoplastics are high risk chemicals because they are small enough to pass through cell membranes as well as the chorion of fish embryos, yet our understanding of their effects on human safety and health remains in its infancy. In this study, by setting the experimental model to human embryonic stem cells (hESCs) and zebrafish embryos, a platform was established to analyze the developmental toxicity caused by nanoplastics exposure from the stem cell stage (embryoid body using hESCs, zebrafish embryo) to the embryonic stage through to the early stage of life (zebrafish from hatching to larvae). In addition, the role of DNA methylation changes in the mechanism of developmental toxicity caused by nanoplastics was studied. The cytotoxicity and pluripotency marker gene expression levels were analyzed by continuously exposing 100 nm of polystyrene nanoplastics (PSNPs) to the hESCs for 5 days. After forming the embryoid body using hESCs, PSNPs were exposed for 14 days to analyze the germline formation marker gene expression level. The zebrafish embryos were also continuously exposed from fertilization to hatching, and the oxidative stress gene expression levels were analyzed. In hatched zebrafish larvae, late developmental effects were studied by measuring malformation and locomotive behavior. At the end of exposure, DNA methylation changes due to PSNPs were screened through global DNA methylation analysis, and the results of global DNA methylation were supported by TET inhibitor assay using hESCs. The PSNPs cytotoxicity on hESCs was not observed, but germline marker gene expression in embryoid bodies was remarkably inhibited, and developmental toxicity, which was not observed in teratogenicity or survival rate in zebrafish, was shown in the locomotive behavior results. Through the results of TET inhibitor assay using hESCs, it was revealed that there is an effect of DNA methylation in the mechanism of developmental toxicity. This suggests the risk of exposure to PSNPs in the early development of life and suggests that the epigenetic effect played a role in the developmental toxicity mechanism of PSNPs.

4.01.V-02 Environmental Risk Assessment of Microplastics for Freshwater Benthic Species Using Strict Quality Criteria and Data Alignment

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Although today we know that freshwater sediments can contain significantly higher microplastic (MP) concentrations compared to the water column, the risks of MP for benthic species have never been quantified. The aim of this study was to assess the environmental risks of MP for freshwater benthic species exposed via sediment using strict quality criteria and ecologically relevant dose metrics. An extensive literature search was first conducted to collect publications reporting measured environmental MP concentrations for freshwater sediments, as well as threshold-effect concentrations for freshwater benthic species exposed to MPs via sediment. The quality of the data was evaluated following published Quality Assurance/Quality Control Criteria (QA/QC). Effect data were rescaled to account for the polydispersity of environmental MPs, and exposure data were rescaled to the full MP size range (1 to 5000 μm). The threshold-effect concentration for each species was calculated for the toxicologically relevant dose metrics volume and surface area of bioavailable MP, assuming effect mechanisms triggered by food dilution or surface area-based particle toxicity upon translocation, respectively. After QA/QC screening and alignment of input data, species sensitivity distributions (SSDs) were constructed for both volume and area as quantitative drivers of effects using chronic no observed effect concentrations of 11 species. For both SSD, we calculated the HC₅, which is the value at which 5% of the species would be affected. Finally, the environmental risks of MP were characterized by comparing the aligned exposure and effects concentrations in a cumulative frequency distribution chart. The derived HC₅ values (95% CI) were 5.3×10^{10} ($6.1 \times 10^9 - 5.0 \times 10^{11}$) and 1.1×10^{10} ($3.0 \times 10^{11} - 5.0 \times 10^{12}$) microplastics/kg sediment dry weight, for volume and area, respectively. All minimum, mean, and maximum MP concentrations measured in freshwater sediments from 37 water bodies were lower than the HC₅ values. Our results demonstrate that after applying strict QA/QC and alignment of exposure and effect concentrations, environmental risks of MP are not likely to occur at current environmental concentrations. Future studies assessing the environmental risks of MP should consider the use of strict quality criteria for the selection of data to avoid biased results caused by poorly design studies. Moreover, aligning exposure and effect data will increase the ecological realism of the studies.

4.01.V-03 How to Investigate Additive-Related Effects From Microplastics?

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When assessing potentially harmful ecotoxicological or toxicological effects of microplastics, researchers are in a quandary: To suit the requirements of interpretable experimental data, they wish well characterized small particles, ideally with a narrow particle size distribution and ideally doped with a well detectable element like Europium. But this scenario is far away from real plastic particles to whom people or environment might be exposed. Microplastic particles that are found in nature show a variety of sizes and shapes – and they do not only contain the polymer itself, but an unknown quantity of unidentified chemicals. Intentionally added chemicals, so-called additives include inorganic particles such as pigments or flame retardants and organic compounds acting as plasticizers or lubricants. Leaching of these unbound components may lead to an exposure of the surrounding environment and eventually cause toxic effects in environmental organisms. To cover both, a realistic testing scenario and the researcher's needs of testing well-known materials including additives, we here suggest the following approach: Based on an extrusion process, an additive loaded polymer of known composition is produced. In a second step, it is cryo-milled, and micron-scaled irregular shaped particles are formed and subjected to weathering and characterisation of additive release as well as ecotoxicity testing. This approach is applicable to both, inorganic and organic additives. To test the proposed approach, a study with Zinc oxide (ZnO) as a model additive has been conducted. Due to its promising properties, ZnO is often applied as flame retardant, heat stabilizer or inorganic pigment for example in low-density polyethylene (LDPE). In contact with water, ZnO dissolves within a short time, where the dissolution velocity increases with decreasing particle size. Moreover, it is assumed that Zinc ions are responsible for toxic effects in fish, daphnia, and others. In our study, a common amount (5 wt%) of a nanoscale ZnO (NanoTek) was homogeneously distributed in LDPE (DOW 410E). An additive-free polymer was processed in the same way. These particles are subjected to further testing with regard to changes in particle properties, the leaching behaviour of Zn depending on weathering, as well as their effects on daphnia and algae. Our results show that this approach is promising to distinguish the effects posed by microplastics and additives.

4.01.V-04 Multigenerational Study of Microplastics in *Folsomia candida*

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Microplastics are the widespread environmental contaminants which found on the mount Everest and in the deep sea. However, there are limited multigenerational study of microplastics though the number of research on soil ecotoxicity of microplastics have been increasing. The purpose of the present study was set to evaluate the multigenerational effects of microplastics in soil arthropods *Folsomia candida*. *F. candida* were exposed by 15.94 µm of HDPE fragments from F0 to F2 generation under maternal or continuous exposure scenario. As results, survival and reproduction rate of maternal and continuous exposed groups were not negatively affected by microplastics. Through the maternal exposure scenario, it was observed that damages in gut barrier were recovered in F1 and F2 generation. However, continuous exposure of microplastics to F2 generation caused inhibitions in adult growth and damages in digestive tract. These results indicated that *F. candida* was not tolerant to continuous exposure of microplastics. *Acknowledgement-This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2020R1A2B5B02001734, 2021R1C1C2012628)*

4.01.V-05 The Effect of Microplastics on the Interspecific Competition of *Daphnia*

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Microplastics (MP) pollution of aquatic environments is currently one of the most intensely studied issues in the field of ecology. Many studies have attempted to estimate the distribution and concentration of MP in various environments and have determined how MP affects their inhabitants. However, much less effort has been undertaken to assess the effect of MP on the inter- and intraspecific interactions between organisms, including interspecific competition in planktonic animals of the genus *Daphnia*. We assumed that the presence of MP could mitigate competition between different *Daphnia* species. To test this hypothesis, we performed 3 long-lasting (40 days) competitive experiments (i.e. each with varying population densities) in the absence (control) and the presence of different types of MP (polystyrene, high-density polyethylene and degradable polyhydroxybutyrate, each of 30 µm in diameter), at daily low algal food supplementation and 3 pairs of *Daphnia*, each pair consisting of a superior and inferior competitor respectively (*D. pulex* and *D. magna*, *D. magna* and *D. galeata* and *D. pulex* and *D. galeata*). In general, the results of our study did not support our predictions, since the negative effect of MP on the population density of competing species was similar to both superior and inferior competitors. The only exception was the effect of polyhydroxybutyrate in the case of *D. magna* and *D. pulex*, where its presence mitigated interspecific competition. This may be attributed to a greater ability of *D. magna* than *D. pulex* to benefit from the presence of additional bacterial food associated with degraded PHB.

4.01.V-06 Laboratory Tests of the Potential Effects of Plastic Leachate on Aquatic Organisms: A Literature Review

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The release of chemicals from plastics is of concern due to the increasing global production of plastics and related increases in amounts of plastics waste in the environment. The potential effects of plastic additives or other weakly bound macromolecules that can leach into the aquatic environment are less well studied than physical effects of plastics on aquatic organisms. We conducted a review of the literature on the use of laboratory toxicity tests to assess the potential effects of plastic leachate on aquatic organisms. We identified, reviewed, and summarized 16 laboratory studies conducted on a variety of plastic types. Crustaceans were the most common taxonomic group tested; others included microalgae, fish, bivalves, snails, and sea urchins. Acute mortality was the most common endpoint examined; others included algal growth and photosynthesis, reproductive and

developmental effects, sublethal metabolic endpoints, and behavior. Concentrations of plastic used to prepare leachate were reported in various ways, including mass of plastics per volume (e.g., mg/L), number of particles per volume, and as area of plastic per volume (e.g., cm² plastic/L). All studies except one reported adverse toxic effects, with mass-based 48-h LC-50 (or EC-50) ranging from 5 g/L to > 250 g/L. Comparison of the results of laboratory tests to field conditions is complicated by differences in reported exposure metrics. For example, concentrations of microplastics (MP) in field-collected surface water samples are often reported as the number of particles per volume of water (e.g., number/L), the number of particles collected per surface area of water sampled by a towed net (e.g., number/m²), or less often as the mass of particles per volume of water (e.g., mg/L). Concentrations of plastics used to prepare leachates in these laboratory studies far exceeded mass-based concentrations of MP in field collected samples as reported in the literature, which contributes to the uncertainty in our understanding of whether the effects of plastic leachate observed in the laboratory are occurring or will occur in the aquatic environment.

4.01 Advancing the field of nano,- and microplastic toxicity exposure scenarios to better support risk assessments (Poster)

4.01.P-Mo198 The Capacity of MNP to Absorb Infochemicals As a Valuable Tool for Refining MNP Hazard Assessment

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Micro- and nanoplastic particles are ubiquitous, their abundance is reported in all parts of the earth, in all bodies of water, all soils and in organisms. Several studies indicate effects of MNPs on organism and ecosystem level, but the modes of actions are not well understood. As most plastics are chemically inert the particle effect itself is discussed not to be the sole reason for affecting organisms. The vector effect - uptake of hazardous chemicals by MNP and the subsequent release in organisms – is also an area for debate. But models indicate that the carrying capacity of the small particles is limited and in combination of the prevalent concentrations in ecosystems, the concentrations transported by the vector effect could be negligible.

Another explanatory approach how MNP influence organisms and ecosystems is the distortion of chemical communication: infochemicals are absorbed by plastic particles and are thus removed from the system. Subtle changes in infochemical concentrations can alter or even distort the message. For example, a small change in hormonal titres can result in an up or down regulation of the biosynthesis of specific proteins; changes in neurotransmitter levels will impact cognitive and perceptive capabilities; changes in the availability of pheromones and predator cues will change an individual's reproductive success and survival probability. In the evaluation of the interaction of MNP with chemicals, the challenge is the immense variety of plastic types and their combination with sizes, shapes, ages, coatings and additives as this increases the numbers of necessary tests. For this reason we have developed an analytical high-throughput system that allows us to evaluate the degree to which MNP remove infochemicals from their surrounding medium.

We hypothesize that the degree of the adsorption depends on MNP-specific traits: For example, larger surface areas should lead to higher absorption rates and the formation of an eco-corona might affect the type of molecules that attach to the particles. To identify MNP traits associated with particularly high absorption abilities, we test various MNPs covering a large variety of MNP traits. MNPs showing high absorption capability with specific infochemicals will be tested in single species bioassays to mesocosms in further studies.

4.01.P-Mo199 Toxicity Interaction and prediction for combined toxicity of Microplastics and Heavy metals on daphnia magna

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Biodegradable plastics have been emerged as alternatives of non-biodegradable plastics, because of property for decomposition in consisted conditions such as light, temperature and microorganisms). Under natural conditions, the decomposition of biodegradable plastics is incomplete, and rather easily forms microplastics. In addition, biodegradable microplastics (BMPs) can easily adsorb more contaminants (e.g. heavy metals, additives) than conventional plastics due to their surface properties, leading more toxicity in aquatic organisms. However, positional effects in affecting the toxicity of BMPs are inadequately documented. In our study, the aim of this study was to investigate the toxic interaction between heavy metals and biodegradable plastics which were decomposed by UV in *Daphnia magna*. BMPs were manufactured by processes with and without process with UV decomposition using BP pellets, and their chronic toxicity to *D. magna* was investigated at lethal (mortality) and sublethal [oxidative stress including reactive oxygen species (ROS), total antioxidant capacity (TAC), and lipid peroxidation (LPO)] levels. As a result of this study, it is expected that microplastics were decomposed by UV will have more adverse effect on *daphnia magna* by adsorbing more heavy metals than conventional plastics (e.g. PE, PS, PET). This toxicity might be associated with the higher bioconcentration of heavy metal achieved by alteration of surface property in BMPs which was decomposed by UV condition than that by conventional microplastics. Overall, this study suggests a significant ecological risk of the toxic interaction between BMPs and heavy metals in aquatic organisms. Our results suggest that biodegradable microplastics can not apply as an alternative to conventional microplastics, and are not an absolute solution.

4.01.P-Mo200 Assessing the Impact of Polystyrene Microplastics on *X. Laevis* Using the Amphibian Metamorphosis Assay (AMA)

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While there is no international consensus on the definition of microplastic (MP), these tiny particles are acknowledged as synthetic polymers with maximum sizes of 5 mm. Among them, polystyrene (PS) is one of the most produced. In the last years, the potential effects of MPs have become an environmental concern and are now considered as emerging contaminants. However, current data on their effects on amphibians is scarce, thus recommendations for increasing the ecotoxicological research in this topic are encouraged. For this purpose, a modified AMA test was developed to evaluate the effects of PS MPs (PMPs; $\leq 200 \mu\text{m}$) administered through the diet on *Xenopus laevis* tadpoles. Two concentrations were selected, 0.84 mg and 8.4 mg PMPs/g food, which corresponded to waterborne concentrations of about 50 (i.e. 1X) and 500 (i.e. 10X) μg PMPs/L, respectively. The 1X was considered as an environmentally relevant concentration, and the 10X a worst-case exposure scenario. The effects on tadpole development (developmental stage), apical endpoints such as whole length (WL), body weight (BW), snout to vent length (SVL), hind limb length (HLL) and histological alterations will be discussed. This work has been supported by Spanish Government Grant RTI2018-096046-B-C21 funded by the MCIN/AEI/ 10.13039/50110001103 and the ERDF.

4.01.P-Mo202 Transgenerational Toxicity of Polyethylene Microplastic Fragments Containing an Ultraviolet Stabilizer to *Daphnia magna*

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Microplastics (MPs; $< 5 \text{ mm}$) are a growing concern due to their small size, wide distribution, and adverse effects. In addition, plastic additives (e.g. photostabilizer, antioxidant, and flame retardant) in MPs can cause additional toxicity to aquatic organisms. However, there are few studies on long-term effect of MPs containing chemical additives. In this study, transgenerational effects of polyethylene MP fragments ($17.35 \pm 5.50 \mu\text{m}$) containing benzophenone-23 (BP-3; $2.85 \pm 0.16\%$ w/w) ultraviolet stabilizer on *Daphnia magna* were investigated across four generations (F0 - F3 generations). Only *D. magna* in the F0 generation was exposed to MP fragments, MP/BP-3 fragments, and BP-3 leachate to identify the transgenerational effect on the F3 generation. Somatic growth (body length) of *D. magna* exposed to BP-3 leachate was recovered in the F3 generation, whereas reproduction (embryonic development and number of offspring per female) was significantly decreased ($p < 0.05$) compared with control. In addition, mortality of *D. magna* exposed to both MP and MP/BP-3 fragments was recovered in the F3 generation, but somatic growth and reproduction were significantly decreased ($p < 0.05$) compared with control. These findings confirmed the transgenerational effects of MP fragment and BP-3 additive on chronic toxicity in *D. magna* across four generations. However, there was no significant difference ($p > 0.05$) in global DNA methylation in *D. magna* among all treatment groups, requiring a gene-specific DNA methylation study to identify epigenetic transgenerational inheritance.

4.01.P-Mo203 Investigation of the Aquatic Hazard Potential of Biodegradable Plastics by Ecotoxicological Studies With Daphnids and Algae

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Since 2021 single-use plastics are banned by the European Union. Therefore, the use and production of biodegradable plastics as an alternative to conventional plastics increases. Since the ecotoxicological impacts of bioplastics are still sparsely researched, bioassays of four new developed bioplastics within the project EU-Bioplastics were performed in this study. Two of the investigated materials are based on poly(lactic acid) (PLA) with a intended application as marine geomaterial and fish crates. In addition, one Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) material applicable as fishing baits and a soft packing material based on poly(1,4-butylene succinate) (PBS) were examined. Acute and chronic leaching and contact tests with the crustacean *Daphnia magna* (OECD202; OECD211) and chronic leaching algae toxicity tests with the marine algae *Phaeodactylum tricoratum* (DIN EN ISO 10253:2006) were performed. The bioplastic concentration was maximum 50 g plastic in 1 L test medium. For chemical validation the test media were analysed by gas chromatography-mass spectroscopy (GC-MS) with regard to HOCs. In acute contact and leaching tests with *D. magna* no immobilisation was observed for PLA, PBS, and PHBV, respectively. During chronic contact tests a significant poor offspring for all materials compared to the negative control was observed. In chronic PLA leaching tests all adult daphnids died between day 7 and day 17 and no offspring was observed. GC-MS analysis of the material showed a contamination of PLA with 2-methylnaphthalene. Chronic PBS leaching tests had the highest amount of offspring with 27 neonates in 21 indicating endocrine effects. In leaching tests with *P. tricoratum* no growth inhibition was observed after 72 hours for PLA and PBS materials, respectively. For PHBV material a growth inhibition with an EC_{50} of 6.3 g plastic per L was observed. No HOC contamination of the media was detected. The results of this study showed that the tested bioplastics cause chronic but not acute toxicity to aquatic organisms. Since bioplastics remain long in the aquatic environment until degradation, the investigation of chronic effects to organisms contributes to the knowledge of bioplastic hazard potential. In addition, to bioassays chemical analysis can contribute to understand why a material cause toxic effects towards organisms, which can help producers to investigate bioplastics which are not harmful for aquatic organisms.

4.01.P-Mo205 Ecotoxicological and Biochemical Impacts of Polystyrene Single Exposure and Adsorbed to Copper on the Freshwater Microalgae *Raphidocelis subcapitata*

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The microplastics issue has become a global concern due to their variety of sources, ubiquity, isolated or combined toxicity to the environment and human health, persistence, and high densities specially in aquatic systems (i.e., freshwater systems).

Microplastics are small plastic particles (< 5 mm) that can adsorb chemical compounds from water or sediment compartments into their surface (e.g., metals, polycyclic aromatic hydrocarbons) and act as a vector of these contaminants. This issue has been more documented for marine environment, while for freshwater systems there is an immense gap in knowledge. For instance, the impacts of these micro-sized particles isolated or in combination with a chemical compound on primary producers are still scarce. Hence, the aim of the study was to evaluate the impacts of polystyrene isolated and adsorbed to copper on the freshwater microalgae *Raphidocelis subcapitata* by assessing the ecotoxicological effects on the growth and nutritional profile (i.e., fatty acids and carbohydrates abundance and composition). The bioassay includes one concentration of microplastics (0.5 p mL⁻¹) and four concentrations of copper (0.055 mg L⁻¹ – concentration below EC₁₀; 0.092 mg L⁻¹ – EC₁₀; 0.155 mg L⁻¹ – EC₂₀; and 0.378 mg L⁻¹ – EC₅₀). All the chosen concentrations are environmentally relevant and these EC_x values were determined in a previous study. Preliminary results suggested that the microplastic and the microplastic-metal combination did not affect the microalgae growth. In fact, when exposed to polystyrene isolated, combination 1 (0.055 mg Cu L⁻¹ + 0.5 p mL⁻¹) and combination 2 (0.092 mg Cu L⁻¹ + 0.5 p mL⁻¹) the microalgae growth was stimulated. Copper isolated bioassay showed a significant growth inhibition at concentration 0.378 mg Cu L⁻¹ (EC₅₀ value). The analysis of the fatty acids and carbohydrates abundance and composition are still ongoing.

4.01.P-Mo206 Effects of High-Density Polyethylene Fragment on the Acute Toxicity on Zebrafish Adults

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Microplastic leads to environmental contamination and causes health problems. Many studies have investigated the toxic effects of microplastic in various organisms, but most studied sphere microplastics. However, there is limited knowledge regarding the adverse effects of microplastics, particularly on fragments. We evaluated the effects of high-density polyethylene (HDPE) fragment which has no additive and Plasticizer, exposure on toxicity and behavior in adult zebrafish. Adult zebrafish were exposed to HDPE (< 25 μm) for seven days at a concentration of 50 ppm. HDPE accumulated in the gastrointestinal organs of the zebrafish. There was no significant behavior change in the exposed group. Significant HDPE exposure effects occurred in reactive oxygen species, acetylcholinesterase (Ache), tumor necrosis factor-α (TNF-α), interleukin 1-beta activity (IL-1β). In addition, gene expression of oxidative stress (Atp13a, Cat, SOD), immune response (TNF-α, IL-1β), neurotoxicity (Ache, Th) were changed significantly. The results of this study provide important insights into the impacts of environmentally relevant (fragmented) microplastics and the molecular mechanism of microplastic-induced toxicity in zebrafish.

4.01.P-Mo207 Toxicity Assessment of Micro-Sized PE in Human Caco-2 and HT-29 Cells

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Microplastics are plastic particles (size < 5 μm) which are primarily a micro-size range or results from degeneration of larger plastic pieces in the environment. Plastics are inert materials, but degeneration can change plastic properties and they can adsorb different types of hazardous substances from the environment. In addition, compounds e.g., phthalates that are used in plastic to improve their properties can be released because of degeneration. However, only little is known how microplastics and plastic associated chemicals affect the human health. Majority of the human exposure to microplastics occurs via ingestion route, and polyethylene (PE) is one of the most abundant microplastics. Therefore, the aim of our study was to evaluate the ability of micro-sized PE to cause harmful effects in human colorectal adenocarcinoma cells. Raw ultra-high molecular-weight PE (Sigma-Aldrich, particle size 40-48 μm) was used. In addition, to investigate the effects of possible dissolved compounds from plastics, extraction with ethanol as solvent was studied. Ethanol-extracted PE samples (PE-EtOH) were prepared by extracting 0.5 g raw ultra-high molecular-weight PE in 10 ml of ethanol in shaker (+37 °C) for three days. After three days, the solution was filtrated and ethanol was evaporated, leaving a PE residue in a glass test tube. PE residue was mixed with 3 ml cell culture medium (100%) and diluted to 75%, 50%, 25% and 10% solutions. In the experiments, human colorectal adenocarcinoma Caco-2 and HT-29 cells were exposed to PE (0.25-1 mg/ml) or PE-EtOH (10-100%) for 48 h. After exposure, cell viability and cytotoxicity were assessed with MTT and lactate dehydrogenase (LDH) assay. Reactive oxygen species (ROS) production was measured with DCFDA (2',7'-dichlorofluorescein diacetate) and cytoplasmic production of superoxide (O₂^{•-}) with dihydroethidium and mitochondrial O₂^{•-} production with MitoSOX. The 48 h-exposure to PE or PE-EtOH decreased cell viability and increased cytotoxicity in both cell lines. Cell viability was decreased in a dose dependent manner with both types of PE used in this study. In addition, both exposures increased ROS production, especially mitochondrial superoxide levels were elevated in all doses in both cell lines. However, effects on cytosolic superoxide production were not observed. In conclusion, micro-sized PE and PE-EtOH decreased cell viability and increased cytotoxicity and mitochondrial superoxide levels on Caco-2 and HT-29 cells.

4.01.P-Mo208 Effect of Polyethylene Microplastic Fragments and Benzophenone-3 Additive on Daphnia magna Population

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Most of the studies reporting toxicity of microplastic (MP) and various plastic additives have focused on adverse effects within individual test organisms. However, population studies of MP exposure are necessary for a more realistic, long-term risk assessment. Therefore, population-level microcosm studies were conducted to evaluate the effect of MPs on *Daphnia magna*. In this study, groups of 10 daphnids were exposed to polyethylene MP fragments (16.68 ± 7.04 μm) containing benzophenone-3 (BP-3; 2.89 ± 0.20% w/w), pristine MP fragment, and MP fragment leachate, respectively (n = 3). The populations were grown for 60 days and observations were made every three days. Somatic growth (body length), reproduction (number of offspring), population growth (biomass, population structure), and bioconcentration of MP and BP-3 were measured.

4.01.P-Mo209 Dependence of Polystyrene Size and Color on Embryonic Zebrafish Mortality

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Hundreds of millions of tonnes of plastic are produced each year, over 80% of which is estimated to end up as pollution in the environment. In response to physical, chemical, and biological weathering these plastics breakdown into and shed micro and nano particles. The ubiquity of small plastic particles in ecosystems around the globe is well documented. The toxicity of plastic pollution cannot be evaluated as a single entity, but instead encompasses a diversity of chemical compositions, colors, morphologies, additive, and sizes. It is necessary to identify which of these features may be most important for toxicity and consequently risk assessment. The existing inconsistency of nanoplastic exposure conditions (e.g. polymer type, concentration, duration, organism) in the literature makes it difficult to draw conclusions about their contributions to toxicity. Overall, the role that particle size and other plastic additives (i.e. colorants), play in overall toxicity of micro and nanoplastic particles is not well understood. Therefore, we designed experiments to investigate the impacts of plastic particle size and color on embryonic zebrafish development and survival. Spherical polystyrene (PS) was selected for these experiments because it is the most widely available nanoplastics for laboratory studies, and is one of the only nanoplastic polymers available in a range of sizes and colors. Embryonic zebrafish were exposed to PS particles of 5 different sizes between 30 nm and 5 mm (n = 8). The colors of the PS nanoplastics included three types of red, as well as orange and blue. Exposures began at 8 hours post fertilization (hpf) and lasted a total of 5 days. Embryos were evaluated daily for mortality and hatching; evaluated at 24 hpf for: mortality, developmental progression, and spontaneous movement; and again at 120 hpf for: mortality, morphological abnormalities, and touch response. Exposure to nano PS resulted in hatch delays and mortality. LC₅₀ values between 167 and 496 mg/L were calculated. Overall, the differing sizes and colors of the PS nanoplastic exposures were found to impact the toxicity, with larger and non-red particles eliciting less toxicity. This suggests that nanoplastic colorant is an important feature and may help to explain contrasting trends in the literature about the effects of nanoplastic size on toxicity.

4.01.P-Mo210 Chemicals From Environmental Aged Plastics Are Toxic to Human Cells

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Humans are exposed to microplastics via ingestion, inhalation, and dermal exposure. Scientific community is working on understanding plastic effects on humans. However, most of the scientific literature study effects of industrial pristine plastics, which can be weakly representative of the environmental exposure. Therefore, in this study we focused on the chemical effects of naturally aged environmental plastics (HDPE, PP, PVC, PA, and PET) collected on the North Atlantic French coast. Chemicals associated to these plastics were chemically extracted from the plastics using the organic solvent dimethyl sulfoxide (DMSO). Organic and metallic compounds were analysed in DMSO extracts and quantified. The human hepatocyte carcinoma cell line HepG2 was selected for its suitability to model liver toxicity including a high ability to metabolize organic contaminants, while the human epithelial colorectal adenocarcinoma cell line Caco-2 was selected to model intestinal exposure. Both cell lines were exposed to DMSO extracts to investigate the effects of chemicals on cell viability, oxidative stress induction and genotoxicity. In addition, to evaluate ER activity, a human breast cancer cell line T47D-Kbluc, stably transfected with a triplet ERE (estrogen-responsive elements)-promoter-luciferase reporter gene construct, was selected. Chemical pattern was polymer-dependent, but PVC was highly contaminated with metals, followed by PA, HDPE/PET, while PP contained low concentrations of metals. For organic compounds, PAHs were mainly found in HDPE and PP, and PVC extracts, as well as organochlorine pesticides. Toxicological assays revealed that cell viability was altered by PVC extracts for both cell-lines, while ROS production increased with PA exposure and DNA damages increased with HDPE, PVC, and PA exposure. Toxicity level is linked to chemical contamination, revealing the importance of chemicals leaching (additives and/or pollutants adsorbed). Despite the increasing literature on the omnipresence of plastics in the environment, the risk of microplastics to human health is still difficult to apprehend. The effects from chemicals (contaminants and/or additives) associated to plastics must be distinguished from other environmental contaminants. Indeed, to evaluate the risk of plastic to human health, it is necessary to quantify the exposure and settle toxicity thresholds above whom risk is significant.

4.01.P-Mo211 Screening and Quantification of Plastic Additives in One-Use Household Items and Cytotoxic Effects in A549 and HepG2 Cell Lines

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Bioplastics derived from renewable biomass sources offering a greener solution for plastic production. However, limited information is still available about their potential environmental impact as well as the impact of related products such as plastic additives. Prospective studies indicated that the toxicity of additives in the new bioplastics may be even at superior levels than in conventional fuel based [1]. This work describes the application of a suspect screening approach to assess the plastic additives in different one-use household items made of bioplastics and petrochemical based plastics. Samples were collected in retail stores including three bags (made of polyhydroxyalkanoates-PHA), spoons, forks, straws, drinking glasses, and plates made of polylactic acid (PLA) and a tap made of polyethylene (PE). The extraction procedure consisted of ultrasonic-assisted extraction with toluene, methanol, and acetone, separately, followed by the suspected screening analysis. The analysis was performed by liquid chromatography coupled to high-resolution mass spectrometry using a heated electrospray ionization source operated in negative and positive conditions [2]. The acquisition was performed in full-scan (FS) and data-dependent acquisition (ddMS²) mode. The

tentative identification criteria were based on mass tolerance (± 2 ppm), the retention time ($\pm 2.5\%$ min), isotopic fit ($>90\%$), fragmentation and mass peak resolution and response [3]. The FS-ddMS² spectra were processed with Compound Discoverer 3.1 to obtain a list of candidates that was further filtered to level 2 matching the experimental fragmentation spectra and comparing them with theoretical patterns. Finally, identification at level 1 was possible for some compounds by means of pure standards confirmation. Cytotoxicity was individually estimated in vitro for MPLs in alveolar cell lines A549 with commonly used petrochemical polymers (PE, PS and PET) and biodegradable polymers PLA and PHB, and in liver cell lines HepG2 for the plastic additives extracted from straw (PLA), tap (PE) and a mix of additives of thirty-eight compounds obtained from available standards. Cells were exposed during 48h ranging from 1 mg/kg to 6.25 μ g/kg (from 100 μ L to 6.25 μ L for straw and tap extracts). The cell viability and the oxidative stress evaluating the Reactive Oxygen Species (ROS) effect were estimated. The first results confirm that the high quantities of plastic additives used in bioplastics to improve the performance of these materials could constitute an important environmental problem. In vitro assays confirmed the ROS effect and cell viability was more impacted by PLA than by common petrochemical plastics in A549 cells lines.

4.01.P-Mo212 Evaluating Direct and Indirect Effects of Nanoplastic Particles on *Daphnia magna* Under Environmental Conditions Using Artificial Wetland Mesocosms

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Plastics pollution is increasing every year in aquatic ecosystems and with it, an ever-increasing number of micro- and nano-sized plastic particles. It has been shown that these particles find their way into the food-chain after being consumed by organisms at lower trophic levels, such as the zooplankton *Daphnia magna*. Previous studies have shown negative effects of nanoplastic particles (NPPs) on reproduction, development, and survival of *D. magna*. However, most studies have only evaluated the effects under small scale laboratory conditions and have not considered them under natural conditions, where different particle interactions may change their toxicity. For this, a mesocosm approach was used to evaluate the effects of NPPs on the behavioral responses and population dynamics of *D. magna*, as well as possible indirect effects through the food source (phytoplankton biomass). *D. magna* individuals were exposed to negatively charged polystyrene (PS) nanoparticles of 88 nm made with a gold core, at a concentration of 0.2 mg/l of PS, for a chronic exposure of 10 weeks under environmental conditions in artificial wetland mesocosms. The swimming behavior was evaluated as the immediate response to ultraviolet (UV) radiation exposure using a three-dimensional tracking method with an *in-vivo* labelling technique. The population dynamics were evaluated through a standardized sampling method from the 5th week of exposure. Lastly, the phytoplankton biomass was measured once a week from the 7th week of exposure. No direct effects from the exposure to NPPs were found on the behavioral responses and population dynamics of *D. magna*. Nonetheless, a trend was observed in the behavior where individuals treated with NPPs seemed to swim shallower during the UV exposure compared to non-treated individuals, resulting also in a reduced refuge demand. As well, the algae biomass was significantly reduced in the treated mesocosms in the last week of exposure compared to the controls. This could potentially lead to a possible indirect effect on *D. magna* in the long run, due to unavailability of the food source. The fluctuations in particle concentration and their interaction with the ecosystem might have played an important role in the toxicity levels observed. This study shows a first glimpse of what could be the effect of NPPs in wetland ecosystems and in key species like *D. magna*.

4.01.P-Mo213 Toxic Effects of Eugenol-Functionalised Silica Particles on the Nematode *Caenorhabditis elegans*

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Functionalisation of silica particles with essential oils components has emerged as a useful strategy to increase these components antimicrobial activity and to overcome some of the limitations of their application to foods, such as high volatility, low solubility or marked sensory properties. These new antimicrobial particles have been proven to be effective preservatives when added to food matrices and as filtering materials for the cold pasteurisation of beverages; however, toxicological studies must be performed to identify possible hazards of their use for human health and the environment. In this work, the nematode *Caenorhabditis elegans* was used as an *in vivo* model to evaluate the toxicological effects deriving from the exposure to different eugenol-functionalised silica particles designed as antimicrobials systems for food applications. For this purpose, the effects of the acute and long-term exposure to three silica particle types (commercial synthetic amorphous silica (SAS), mesoporous silica (MCM-41) microparticles and MCM-41 nanoparticles) at three concentrations (0.2, 1 and 5 mg/mL), either bare or functionalised with eugenol, were evaluated on different biological parameters of *C. elegans*, such as survival, reproduction, locomotion behaviour or growth. Acute exposure to the bare and EOCs-functionalised silica particles did not affect nematode survival or reduced the locomotion behaviour, but affected worms' reproductive capacity. Specifically, 24 h exposure to eugenol-functionalised MCM-41 micro and MCM-41 nano at the two highest tested concentrations reduced the number of larvae laid per worm, and all the tested concentrations for the bare and eugenol-functionalised materials significantly reduced the egg number per worm. Moreover, long-term exposure to the different bare and EOCs-functionalised silica particles caused the inhibition of both, brood size and nematode growth. The multivariate analysis showed a positive correlation between the physico-chemical properties of particle's such concentration and functionalisation yield with different reproductive toxicity parameters in *C. elegans*. Our findings give an insight of the potential toxic effects deriving from the exposure to the eugenol-functionalised silica particles and may be useful for the design of safer new materials for consumers applications. **Acknowledgement:** Spanish Government (Project RTI2018-101599-B-C21 (MCUI/AEI/FEDER, EU)), the Generalitat Valenciana (grant agreement no. ACIF/2016/139) and the *Caenorhabditis elegans* Center (CGC) funded by the NIH Office of Research Infrastructure Programs (P40 OD010440).

4.01.P-Mo214 Challenges in Developing a Standardized Risk Assessment for Micro- and Nanoplastic Particles

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Micro- and nanoplastic particles (MNPs) are highly complex pollutants. Each particle is characterized by a unique set of traits. These traits include the polymer type that forms the particle (e.g., poly ethylene, poly styrene, etc.), a set of particle-specific characteristics (e.g., shape, size, surface structure, surface charge etc.) and a variety of traits related to plastic-associated chemicals, each of which has its own molecular structure and properties. Furthermore, most of these traits can change over time through degradation processes and weathering. An eco-corona formed on the surface of the particles may additionally alter or influence all above mentioned properties, which in turn may affect the environmental behaviour of MNPs and their interaction with organisms. This complexity makes the development of a standardized hazard assessment scheme for MNPs challenging, because neither the one-substance-one-test approach currently used in regulatory hazard assessment of chemicals, nor an approach focussing solely on polymer or particle properties can represent this complexity sufficiently.

We discuss the difficulties that MNP complexity imposes on establishing a standardised hazard assessment scheme and argue that MNP hazard assessment needs to be guided by deconstructing MNPs into their traits and identifying those traits that are associated with particularly toxic outcomes. Using a sample dataset on MNP toxicity to *Daphnia* spp., we illustrate how the identification of traits and combinations of traits as toxicity drivers can be used to inform hazard assessment. Based on these considerations, we emphasize that the identification of traits associated with toxicity is similarly essential for guiding the focus and alignment of MNP exposure, and consequently, risk assessment.

4.01.P-Mo215 Ecotoxicological Impact of Microplastic Chemical Leachates From Common Consumer Products on the Early Life Stages of Atlantic Cod (*Gadus morhua*)

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Synthetic polymer-based materials are omnipresent in aquatic environments, where weathering and degradation processes can lead to their progressive fragmentation and the leaching of polymer-associated chemicals. The current study assessed chemical composition and toxicity of microplastic chemical leachates on early life stages of Atlantic cod (*Gadus morhua*). Microplastic particles derived from common consumer products such as balloons (BAL), car tire rubber (CTR) and dishwashing gloves (DG) were used to produce leachate stock solutions (100 g/L) which were then diluted with seawater to 5 different leachate exposure concentrations (from the highest to the lowest: C1-C5 where C1=100% and C5=6.3%). In addition, 2 CTR-specific chemical components, benzothiazole (BT) and 6PPD-quinone (6PPD-q) (degradation product of *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine; 6PPD), were assessed, similarly to the plastic leachates, at 5 concentrations ranging from 15 mg/L (C1) to 0.2 mg/L (C5) for BT and from 50 µg/L (C1) to 0.08 50 µg/L (C5) for 6PPD-q. Considered endpoints included survival and mortality, hatching success and developmental alterations assessed by using morphometric measurements. Non-target GC-MS analyses combined with mass spectral library matching (>80% similarity) revealed a number of organic compounds in the leachates, representing antioxidants, antimicrobials and plasticizers. CTR and DG leachates had the highest content of tentatively identified organic additives. The DG and CTR leachate exposure led to lower hatching success and increased mortality from C3 to C1 compared to BAL leachates, which did not cause any significant effect. The hatching success and survival of embryos was significantly reduced after exposure to CTR leachates and benzothiazole (C2-C1), while no significant effect was observed in 6PPD-quinone treatments. Embryonic development was impacted by exposure to CTR and 6PPD-quinone at the highest concentrations for both treatments, resulting in smaller larvae at hatch. Larvae exposed to the highest 6PPD-quinone concentration exhibited a high degree of spinal deformation (scoliosis). This study indicates that microplastic leachates can affect survival and development of sensitive life stages in cod, where different chemicals caused different effects. Further research should be directed towards mechanistic-assessment of the toxicity driving components in microplastic leachates for a better understanding of the overall impact of plastic-associated chemicals on aquatic species and to identify key polymer-associated chemicals for possible mitigation measures.

4.01.P-Mo216 Microplastics Inhibiting Sediment Reworking ACTivity and Organic Matter Processing by Tubifex tubifex Bioturbators

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The impact of microplastics on bioturbation activities and biogeochemical cycles are poorly acknowledged. Therefore, in an attempt to better understand this subject, we conducted two mesocosm experiments on deposit-feeders, *Tubifex tubifex*, to investigate the effects of sediment contamination with a heterogeneous mixture of variable sizes, morphologies, colors and different polymer types of microplastic on bioturbation activity of *tubificid* worms. In the first experiment, and after twenty-one days, the sediment reworking activity of *tubifex* bioturbators exposed to microplastics was reduced by almost 4 folds and a half in comparison to worms presented in the absence of microplastic. In the second experiment, after exposing the *tubifex* worms to the same concentration of microplastics as in the first experiment (100 mg.Kg⁻¹) but for longer-duration, for seventy-seven days, the biogeochemical cycles triggered by *tubificid* worms (CO₂ fluxes and NO₃⁻ efflux) were significantly reduced. Our results indicate that adverse impacts are caused on bioturbation level which reduce both bioturbator sediment reworking activity and their role in stimulating the biogeochemical cycles at the water-sediment interface.

4.02 Antimicrobials and antimicrobial resistance under the One Health Approach (Poster)

4.02.P-Mo217 Development of an Analytical Method for the Analysis of a Variety of Antimicrobials in Horse Manure and Earthworms

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The massive use of antimicrobials in veterinary treatments during the last decades has raised concerns regarding the occurrence of antimicrobials in the environment and the consequent proliferation of antimicrobial-resistant genes (ARG). The application of manure and slurry coming from treated livestock as fertilizers has contributed to the accumulation of these pharmaceuticals in agricultural soils, threatening the welfare of non-target organisms. Earthworms, representing the 60-80 % of the total soil biomass, have the ability to bioaccumulate soils contaminants via ingestion of contaminated organic matter as well as by dermal route, therefore, they may be the ideal organisms for identifying antimicrobials in the food web. Although toxicity studies of classical pollutants have been performed with these organisms, there is still scarce information regarding antimicrobial accumulation and toxicity. Within this context, in this work an accurate analytical method was developed for the analysis of tetracyclines and sulfonamides antimicrobial families in horse manure and earthworms. The method consists on a QuEChERS based extraction followed by a solid-phase extraction (SPE) clean-up step, using Oasis HLB sorbent, and the analysis was performed by liquid-chromatography-triple quadrupole tandem mass spectrometry (LC-QqQ-MS/MS). For method optimization, the loading sample volume was studied in the clean-up step for both matrices, as well as the quantity of the sample to be weighted in the case of the manure. The method was validated and applied to horse manure samples and *Eisenia fetida* earthworms that had been exposed to known (0, 5, 10, 50, 100, 500, 1000 mg/kg) sulfamethazine and tetracycline concentrations by different exposure pathways and times. Furthermore, possible toxic effects of antimicrobials in earthworms were evaluated by three different OECD Standard Toxicity Tests: a filter Paper Contact test (OECD-207), an Artificial Soil test (OECD-207) and a Reproduction test (OECD-222).

4.02.P-Mo218 Antibiotic Uptake by Spinach (*Spinacia oleracea*) and Radish (*Raphanus sativus*) - Distribution Between Above- and Belowground Plant Fractions, Soil, and Soil Pore-Water

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Antibiotics are extensively used in animal husbandry for disease treatment, illness prevention and growth promotion. The major route for excreted antibiotics and their metabolites to croplands is via the application of animal manure fertilizers. Once applied onto agricultural soils, they can be taken up by plants and accumulate into edible tissues. The extent to which this process takes place depends on the physicochemical properties of the antibiotic compounds, soil sorption potential, crop species and environmental conditions. The objectives of this study were to evaluate the uptake of five antibiotics to spinach (*Spinacia oleracea*) and radish (*Raphanus sativus*) and their distribution between roots, leaves, soil and soil pore-water. A mixture of five antibiotics from different antibiotic classes; clarithromycin (macrolide), sulfamethoxazole (sulfonamide) and the co-applied trimethoprim, chlortetracycline (tetracycline) and enrofloxacin (fluoroquinolone), were applied to the soil in four concentrations ranging from 0 mg/kg to 10 mg/kg (dry weight). Following antibiotic addition, the crops were grown for 6 weeks under controlled environmental and climatic conditions, after which they were harvested, and samples were collected for soil and pore-water analysis. Soil and soil pore-water samples were collected at three additional time points (2 days, 1 week and 3 weeks) from the start of the experiment. Delayed sprouting and reduced biomass production were observed for radishes exposed to the highest antibiotic treatment. The antibiotic analysis, to be performed, will show the uptake of antibiotics to the plants, their distribution between above- and belowground plant fractions, soil, and soil pore-water.

4.02.P-Mo219 The Differential Effect of Triclosan Concentrations on the Emergence of Resistant Bacteria

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The influence of antimicrobials (antibiotics and biocides) on antibiotic resistant has been broadly studied in the clinic settings, but not in the environmental surroundings. Antimicrobials can be found in many environments at different concentrations, such as wastewater, wastewater treatment plants (WWTPs) and surface water, because of their use. The objective of this work was to study the impact of triclosan on a model bacterium mimicking what could happen on those environments, carrying out an evolution assay. Triclosan, a biocide presents in household products, such as gels, soaps or toothpaste was assessed on a bacterial model *Escherichia coli* for 28 days to different quantities, 1/10 and 1/50 of the minimal inhibitory concentration (MIC) of *E. coli* and increasing concentrations above its MIC. Triclosan susceptibility phenotype analysis showed higher levels of resistance in populations evolved in the presence of high amount of triclosan, which appear early in time and gradually increase over time. The evolved population in the presence of low amount (1/50 MIC) did not show or showed low triclosan resistant phenotype (1/10 MIC), and longer time was required to acquire resistance without subsequently further changes. In both cases, evolved population displayed little or no increase in resistance to antibiotics (cross-resistant). Despite the difference in phenotype, the acquisition of resistance had a negative effect on the growth, being in both cases lower than in the wild type. Evolved populations were sequenced looking for the mutations responsible for the phenotype, finding different mutations as a function of the selective pressure exerted. Among the mutations found, mutations in *fabI* gene, described previously as the target of triclosan, and in *emrR* gene, transcriptional repressor of multidrug efflux transporter EmrAB, were identified under supra-inhibitory concentrations. Only mutations in *emrR* gene but not in *fabI* gene were found in evolved population under low biocide pressure. The low cross-

resistance to antibiotics and the fitness cost of evolved populations suggests a specific effect of triclosan and the prospect that it may not be maintained over time without selective pressure.

4.02.P-Mo220 Is the Current Lower-Tier Threshold Concentration for Veterinary Antibiotics Sufficiently Protective for Freshwater Biofilms?

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The current regulatory framework for antibiotics assumes that concentrations below 1 µg/L are not posing a risk to aquatic biodiversity and ecosystem functioning. Recent studies demonstrate that aquatic microorganisms (fungi, bacteria, archaea) forming freshwater biofilms may have a very high sensitivity to low concentrations of antibiotics. Furthermore, some studies demonstrate that concentrations below regulated thresholds can contribute to the development of bacterial resistance. The aim of this study was to evaluate whether the exposure to a realistic environmental concentration range of an antibiotic used in aquaculture (including the 1 µg/L threshold) may impact the bacterial community structure of freshwater biofilms and whether it can contribute to the selection of bacterial resistance. An evaluation of how the bacterial community and the resistome (identified genes for antibiotic resistance) may change/recover after the cessation of exposure was also performed. For that, a microcosm experiment was conducted under laboratory conditions where biofilm structures formed for a period of 14 days in a clean river (Sorbe River, Guadalajara, Spain) were exposed to 5 treatments with different flumequine concentrations (Control, 0.1, 1, 10 and 100 µg/L). The biofilms were exposed to the antibiotic for 7 days to evaluate the effects of the antibiotic and next, the biofilms were transferred to microcosms containing a clean water medium (river water without antibiotic) and cultivated for another 7 days to assess post-exposure biofilm changes and potential recovery. The analysis to assess the antibiotic impacts on the bacterial community composition was performed by DNA extraction and sequencing. For the resistome analysis, real-time PCR analysis was performed using a primer set targeting genes conferring resistance to antibiotics, heavy metals and genes encoding mobile genetic elements associated with ARGs. Analysis of Colony Forming Units (CFUs) was performed in R2A plates without and with 2 mg/L of flumequine by scraping the biofilms before and after exposure to antibiotics and after the recovery time. All results will be put in context with the 1 µg/L threshold established by the current VMP ERA regulations and the question as to what extent such concentration threshold is protective for the bacterial structure of the biofilm and its resistome will be explored.

4.02.P-Mo221 Chemical Stress As a Silent Driver of Climate Change? Antibiotics Increase Methane Production in Freshwater Sediments

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The increasing concentration of atmospheric greenhouse gases is the main driver of current and projected climate change, and therewith of serious public concern for human health and the environment. Methane (CH₄) emissions from freshwater ecosystems (wetlands and inland waters), as the largest and most uncertain source of atmospheric CH₄, are rising, leading researchers to investigate environmental factors and climate change feedbacks to explain this increase. Despite their omnipresence in freshwaters, knowledge on the impact of anthropogenic chemical stressors (e.g., antibiotics) on methanogenesis is lacking. To tackle this knowledge gap, we examined the effects of antibiotics on CH₄ production and microbial communities in natural freshwater sediments. Samples were incubated under anaerobic conditions with a five-component antibiotic mixture at four levels (from 0 to 5000 µg/L sum concentration) for 42 days. Weekly measurements of CH₄ and CO₂, as well as their compound-specific ¹³C, showed that the rate of methanogenesis was increased by up to 94% at 5000 µg/L and up to 29% at the field-relevant concentration (i.e., 50 µg/L). Metabarcoding of the prokaryotic 16S rRNA region suggested that effects of antibiotics on prokaryotic community level (i.e., species composition) may partially explain the observed differences in CH₄ production rates. Despite the intricacy of transferring experimental CH₄ production rates to field conditions, our study showed a proof-of-concept that antibiotics can have adverse effects on greenhouse gas emissions by affecting methanogenesis in polluted freshwater ecosystems.

4.02.P-Mo222 Effects of erythromycin on juvenile rainbow trout microbiome and fitness

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Small concentrations of antibiotics, such as erythromycin, are typically discharged continuously in effluents of WWTP. Erythromycin, which is a broad-spectrum macrolide antibiotic, is used to treat bacterial infections and is one of the antibiotics most commonly used in both human and veterinary medicine. These discharged antibiotics and their pseudo-persistence could potentially result in negative effects on ecosystem health, including condition and health of fishes. Fish serve important functions and roles in ecosystems and can be used as integrative, sentinels of aquatic ecosystems. Gut microbiomes of fish serve significant roles in overall condition of hosts, and dysbiosis could have important implications for health of host fishes. Results of the study reported here investigated responses of the gut microbiome to exposure to dietary erythromycin using 16S rDNA amplicon sequencing and quantitative PCR (qPCR). Profiles of short-chain fatty acids (SCFAs), which have essential roles in health of fish, were measured in blood serum of exposed fish using targeted metabolomics. Erythromycin was hypothesized to decrease abundances of microbes and shift gut microbiome community composition of exposed fish, relative to that of the unexposed control individuals. Due to declines in anaerobic microbe abundance and changes in gut microbial structure, absolute and relative concentrations of serum SCFAs or changes in SCFAs profiles were also expected. This could lead to adverse effects on host fish

due to changes in energy metabolism, decreased maintenance of gut and immune homeostasis, and fluctuations in neural signaling. Results of this study provide insight into potential effects of changes in microbiome communities and overall function due to antibiotic exposure, leading to altered fitness.

4.02.P-Mo223 Occurrence of Antibiotic and Ecotoxicological Risk Assessment From Three Hydrographic Basins Receiving Urban Wastewater Effluents

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The extensive use of antibiotics in the past decades have recently raised global public health concerns due to the growing problems of antimicrobial resistance proliferation. Wastewater treatment plants (WWTP) have been identified as one of the main sources of these active pharmaceuticals to the aquatic environment, as antibiotics have been reported to not get properly degraded in the most common water treatments implemented and are thus released in the environment. In this study, the occurrence of antibiotics was evaluated in three riverine basins of the Basque Country (NE of Spain) impacted by WWTP effluents gathering urban, industrial and hospital wastewater discharges. Four sampling campaigns were carried out in November 2019, January and September 2020 and January 2021. Water samples were taken in three sampling points established upstream and downstream WWTPs discharge and in the point directly affected by wastewater effluents. In addition, an integrated 24h sample from the WWTP effluent was analyzed in every campaign. Thirty-one antibiotics were screened in the water samples after a solid phase extraction and liquid chromatography coupled to high resolution mass spectrometry analysis (LC-HRMS qOrbitrap). A total of 18 antibiotics were detected in WWTP effluents and river water, including macrolides, fluoroquinolones, beta-lactams and sulfonamides. The environmental ecological risk of pharmaceuticals was assessed based on calculated Risk Quotients (RQ) in the wastewater effluents and surface water from the concentration data found in the samples. According to our data, four antibiotics (namely azithromycin, amoxicillin, norfloxacin and ciprofloxacin) posed a high risk on the aquatic environment in the studied areas. Temporal trends and possible consequences will be evaluated according to the reported data, willing to enhance the antibiotics assessment in fresh water ecosystems. *Acknowledgement:* This work was financially supported by the Spanish Ministry of Science and Innovation through the RTI2018-093989-B-I00 project.

4.02.P-Mo224 Persistent Carbapenemase-Producing *Escherichia coli* and *Klebsiella oxytoca* in Wastewater and Associated River

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Increasing resistance to carbapenem antibiotics in *Enterobacteriaceae* is threatening the ability to treat multidrug-resistant infections. Carbapenemase-producing *Enterobacteriaceae* (CPE) are increasingly reported in healthcare settings worldwide, however, their environmental distribution and persistence are unclear. The present study aimed to characterize CPE from a wastewater treatment plant (WWTP) and the recipient aquatic environment in central Sweden during 2015-2018 and to identify potential species as persistent reservoirs of carbapenemases genes. The bacterial isolates were recovered from wastewater and downstream river water samples using chromogenic media selective for carbapenem-resistant Gram-negative bacteria, identified by MALDI-TOF and carbapenemase production was confirmed using a hydrolysis assay. The genomes of CPE were sequenced and the genetic relatedness was determined using multi-locus sequence typing, average nucleotide identity (ANI) and core-genome phylogenetics. The isolates were clustered based on their genotypic and phenotypic resistance profiles. A total of 43 CPE recovered belonged to three different species; *Escherichia coli* (n=32), *Klebsiella oxytoca* (n=8) and *Raoultella ornitholytica* (n=3), all regarded as potential human pathogens based on genetic virulence markers. The majority of CPE were isolated from wastewater (n=37), while 6 were detected from the WWTP effluent recipient river. The most prevalent carbapenemase was *bla*_{OXA-48} (*E. coli*, n=20; *R. ornitholytica* n=3), followed by *bla*_{OXA-181} (*E. coli*, n=10) and *bla*_{VIM-1} (*K. oxytoca*, n=6). The results revealed that 6 *bla*_{VIM-1}-encoding *K. oxytoca* isolates from different years were genetically identical (ANI 99-100%) and belonged to the sequence type ST172. Similarly, some *bla*_{OXA-48}-harboring *E. coli* (ST38) isolated in 2015 were genetically identical to those detected in 2017 and 2018. Since most of these CPE were recovered from wastewaters, it is concluded that they are prevalent in the community and/or are successfully adapted for survival in the wastewater system. Furthermore, successful dissemination of CPE in aquatic environments of Sweden, a country with a low clinical prevalence of antibiotic resistance is present, therefore, advanced wastewater treatment is required to further reduce the global spread of CPE.

4.02.P-Mo225 The Profiles of Antibiotic Resistance in the Groundwater of Maozhou River Basin in Shenzhen, China

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Groundwater has been affected dramatically by anthropogenic activities which leads to the increase of antimicrobial resistance bacteria (ARB) and antimicrobial resistance genes (ARGs). However, to a large extent the prevalence of those in groundwater is still not clear. Here, we investigated the antimicrobial resistance in groundwater from Maozhou River basin (Shenzhen, China). *E. coli* strains were selected as the representative bacteria species due to its close relationship with the intestinal health of human and animals. In total, 78 strains of *E. coli* were isolated from the investigated region (20 strains from 10 monitoring wells and 50 strains from 10 domestic wells). Their antibiotic resistance phenotypes and genotypes were analyzed. Antimicrobial susceptibility tests were performed by using 8 different classes of antibiotics and 18 ARGs subtypes and 3 mobile genetic elements (MGEs) were detected by PCR. The results showed that 71.8% of the isolated *E. coli* were antibiotic resistant strains, with the highest resistance rates to ampicillin (66.7%), followed by tetracycline (32.1%). Six strains of *E. coli* were multidrug-resistance bacteria (MDR) which showed resistance to more than 3 antibiotics. Nine ARG subtypes (*bla*_{TEM}, *aadA*, *cfr*, *ereA*, *qnrD*, *qnrS*, *sulI*, *sul2*,

tetC) and two MGEs (*Int1* and *Int2*) were detected. About 59.2% of these isolates carried at least one of the target ARG or MGE. The most frequently detected genes were *blaTEM* (28.2%) and *qnrD* (26.9%). The results from this study indicated that the prevalence of antibiotic resistance bacteria and antibiotic resistance genes is quite high in groundwater of this region which may pose a potential health risk to local people who use the water directly.

4.02 Antimicrobials and antimicrobial resistance under the One Health Approach (Poster Corner)

4.02.PC-Mo07 One Health Perspective: A Local Study on the Spread of Antibiotic-Resistant Pathogens in Aquatic Ecosystems

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The European rules for assessing the water quality of drinking and bathing water fixed mandatory requirements for the health of consumers and bathers. And they are highly precautionary. At the same time, the assessment of surface water is based on the analysis of biological elements expressed as an ecological quality ratio. It is not included parameters for assessing risk for human health. The presence and spread of pathogens and drug-resistant strains in the environmental sector are underestimated due to the lack of a dedicated surveillance system. Even if the last represents a crucial point in their transmission route to animals and humans. This lack of information and data from the environmental sector probably means the most significant gap in controlling and preventing infectious diseases from a One Health perspective. This work's objective was to search pathogenic microorganisms, antibiotic-resistant too, of sanitary and veterinary interest in surface waters. The study area was an urban area with highly impacted aquatic ecosystems. The pathogens searched were *Escherichia coli*, *Salmonella* spp, *Yersinia* spp. and *Staphylococcus* spp in raw water sampled in three sites chosen to represent the pollution gradient. In vitro tests were performed to detect the presence or absence of resistance of bacterial strains isolated against a set of common antibiotics used to treat human and animal infections. The results have highlighted the presence of target pathogens studied in the aquatic ecosystem and the presence of multi-resistant strains. The direct and indirect reuse of contaminated surface water, such as agriculture, fishing, breeding, and recreational activities, represents an unexplored or scarcely poorly known sector where pathogens escape to the control and prevention system. In conclusion, even if it is a local study, the results represent a promising environmental contribution in implementing the prevention actions plan according to the One-health perspective.

4.02.PC-Mo08 Bioaccumulation of Antibiotics and Resistance Genes in *L. Sativa* Leaves Under Manure and Digestate Application and Their Effects on Microbial Community

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The frequent practice of employing zootechnical waste (e.g. manure) for soil fertilizer can be a source of antibiotics (ABs), antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs), such as sulfonamides and fluoroquinolones. ABs and ARGs from agroecosystems can be accumulated in plants and eventually pass through food chain to animals and humans which feed on fresh vegetables. Sulfamethoxazole (SMX), enrofloxacin (ENR) and ciprofloxacin (CIP) are commonly used in both human and veterinary medicine, and their residues are among the most commonly antibiotics found in the environment. The detection of antibiotic residues in crops amended with organic fertilizers has received increasing attention. In a review of the incidence and uptake of several antibiotics in edible crops, Pan and Chu reported that antibiotics can be taken up by plants through water transport and passive absorption. In this context, antibiotic plant uptake from soils spiked with fixed concentrations can improve knowledge on this issue. In this work, the bioaccumulation of spiked SMX, ENR and CIP (7.5 mg/kg) and ARG occurrence in *L. sativa* leaves grown on a soil amended with manure or digestate have been investigated. Moreover, the leaf microbial community was also evaluated sequencing its 16S rRNA gene. Interestingly, CIP and ENR were found to bioaccumulate in the leaves of *L. sativa*, while SMX was not bioaccumulated (BAF < 1). Moreover, *L. sativa* leaves grown on soil amended with manure significantly bioaccumulated more antibiotics than those grown on soil amended with digestate; in line with the latter result the highest values of ARGs were also found in this condition. The most abundant genes were *sul1* and *sul2*, although SMX concentrations were the lowest ones. Interestingly, in the leaves grown on amended manure soil, a correlation between the highest abundant genes (*sul1*, *sul2* and *int11*), all antibiotics and several bacterial genera were found. Among them *Escherichia*, *Shigella*, is known for carrying ARGs and including several human pathogens species. The presence of some potential human pathogens (i.e., *Nocardia*) has also been found in digestate. The presence of antibiotic residues and ARGs in *L. sativa* leaves, raises several concern because it is consumed by humans.

4.02.PC-Mo09 An Accurate Analytical Method for the Simultaneous Determination of Tetracyclines and Sulfonamides in a Variety of Vegetable Samples

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The massive use of antibiotics in cattle industry has led them to reach the food chain through the accumulation in plants and vegetables of frequent human consumption, among others. Consequently, major concern has arisen in the last decades regarding food safety and human health, not only due to the possible effects after ingestion of contaminated food products, but also since transmission of antibiotic-resistant bacteria could occur. In this work, an accurate analytical method consisting of a QuEChERS

based extraction and detection by liquid-chromatography-triple quadrupole tandem mass spectrometry (LC–QqQ-MS/MS) was developed and validated for the simultaneous analysis of four tetracyclines (TCs) and five sulfonamides (SAs) in a variety of vegetables (lettuce, onion, tomato, and carrot). In the extraction, the effect of the addition of different extraction salts (Na_2SO_4 or MgSO_4) was evaluated, concluding that using Na_2SO_4 as dehydrating agent significantly improved the recoveries for TCs and SAs in lettuce and SAs in onion, while no significant differences were noticed in the rest of the matrices. Due to the lack of selectivity of QuEChERS, dispersive-solid phase extraction (dSPE) and solid phase extractions (SPE) clean-up approaches were also evaluated, concluding that Oasis HLB based SPE provided the best overall results in terms of analyte recovery and matrix effect suppression. The evaporation step and the injection solvent were thoroughly evaluated too and were eventually fixed at evaporation to 1 mL in glass test-tubes and acetonitrile:oxalic acid (aq, $0.01 \text{ mol}\cdot\text{L}^{-1}$, pH 2) 1:1 (v/v) mixture, respectively. Calculated apparent recoveries for most of the target analytes were over 60 % with adequate precision in all the matrices ($\text{RSD} < 30 \%$), while the procedural limits of quantification (LOQ_{PRO}) values remained below $4.7 \text{ }\mu\text{g}\cdot\text{Kg}^{-1}$ for TCs in all vegetables, except for lettuce (up to $12.8 \text{ }\mu\text{g}\cdot\text{Kg}^{-1}$), and $3.0 \text{ }\mu\text{g}\cdot\text{Kg}^{-1}$ for SAs in exception of SDZ ($8.0\text{-}9.4 \text{ }\mu\text{g}\cdot\text{Kg}^{-1}$). Finally, the method was applied to lettuce, onion, tomato, and carrot samples from both ecological and conventional agriculture for quantifying parent antibiotics and degradation products.

4.02.PC-Mo10 Antimicrobial Resistance Genes and Mobile Genetic Elements in River Sediments of Wastewater Effluent-Receiving Areas

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There is a growing concern about the fate of antibiotics in the environment since recent studies have evidenced that sublethal concentrations of those products may act as a selective pressure for the acquisition of *antimicrobial resistance (AMR)* and dissemination of *antibiotic resistance genes (ARG)*. Moreover, those molecules do not appear individually but as complex mixtures with other chemical substances, as it is the case of metals which seem to promote co-selection between *metal resistance genes (MRG)* and ARGs. Here, we highlight the relevance of local inputs of wastewater treatment plants (WWTP) as important sources of these pollutants in aquatic systems. To do this, a systematic monitoring was conducted in three wastewater effluent-receiving rivers, where composite sediment samples and surface water samples were collected in non-impacted (basin headwaters) and impacted sites (downstream) during two monitoring campaigns: November 2019 (wet season, high water period) and September 2020 (dry season, low water period). Both relative (normalized to *16S rRNA* gene) and absolute abundance of 9 ARGs, 4 MRGs and 3 MGEs (*mobile genetic elements*) in sediment samples were quantified using quantitative PCR. In addition, physicochemical characteristics and pseudo-total metal contents were also measured in those river sediments samples and antibiotic concentrations were detected in both sediment and water matrices as well as in 24-h composite effluent samples taken in those facilities. Quantitative analysis revealed that correctly quantified genes appeared in all monitored sites regardless of sampling season, though absolute abundances were significantly higher in dry season as compared to wet season. Regarding spatial distribution patterns, significantly higher relative abundances of most quantified genes were generally found in downstream sites as compared to those in basin's headwaters. The relationship between target genes and environmental parameters evidenced significant correlations of most measured metals in surface sediments and antibiotics in water samples with relative abundances of some MGEs, thus indicating selective pressure of this pollutants can promote HGT. Moreover, correlations between antibiotics and particular ARGs were also observed, though not necessarily with paired antibiotics. Finally, MGEs showed significant correlations with ARGs and MRG suggesting that HGT may have enhanced resistance acquisition.

4.02.PC-Mo11 Multiple Tools for Antibiotics and AMR Characterisation in Aquatic Ecosystems - a 2-Year Monitoring Study

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The ubiquitous presence of antibiotics exerts a selective pressure on microbial communities leading to the acquisition and dissemination of antimicrobial resistance (AMR) in the environment. To better understand antibiotics & AMR dynamics in the aquatic environment and to identify hot spots of accumulation, we conducted a large field study consisting in 8 sampling campaigns over 2 years on 4 stations belonging to French regional observatories and presenting contrasting levels of pharmaceuticals: 2 on the Arve river belonging to Sipibel observatory and 2 on Lake Geneva belonging to the Observatory on Lakes. On each sampling campaign, the following parameters were determined: (i) antibiotics levels in water, sediments and periphyton; (ii) AMR in periphyton and sediments using various detection techniques: resistance genes & integrons quantification, evaluation of tolerance acquisition *via* a PICT (Pollution Induced Community Tolerance) approach; (iii) antibiotics biodegradation potential of microbial community from sediments (by radiorespirometric measurement); (iv) diversity of bacteria and diatoms in periphyton and sediments; (v) physico-chemical parameters. While microbial resistance to antibiotics is commonly assessed by quantifying resistance genes or isolating antibiotic resistant bacteria, AMR can also be estimated by measuring the acquisition of antibiotics tolerance at community level, following a PICT approach. In our study, periphytic microbial communities from the Arve river were found to have a higher tolerance to the tested antibiotics (ciprofloxacin, ofloxacin, sulfamethazine and erythromycin) than communities from Lake Geneva, in agreement with the expected levels of contamination. In addition, in some cases, a higher tolerance was also found at stations close to waste water treatment plants (WWTP) effluents

than in upstream/protected stations. For example, periphytic microbial communities collected downstream the WWTP on the Arve river were generally found to have a higher tolerance to ciprofloxacin than the upstream communities. Comparing whole community tolerance to other classical indicators of AMR and to antibiotics levels in the aquatic ecosystems allows us to better understand the interconnection between pharmaceutical exposure, *in situ* tolerance and genetic potential for A

4.02.PC-Mo12 Modelling the Distribution of Antibiotics in the Global River System

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Significant concentrations of antibiotics in rivers may lead to the gradual development of drug-resistant bacteria, among many other potential impacts. Still, there is a lack of observational data in the field since these substances are not typically included in routine monitoring programs, especially in developing regions. In this work, we developed a model to estimate the emission of various antibiotics and their subsequent transport in river networks at high spatial resolution (500 m) and global scale, enabling first-time estimates of the surface-water concentrations of these compounds for virtually any river in the world. A key component of this research is the integration of two novel datasets in the modeling approach: (1) the average levels of consumption of 84 different antibiotics for each country in the world, which are used to estimate the release of the antibiotics in each region; and (2) a global database of 58,502 wastewater treatment plants (WWTPs); which includes enough information to geo-locate point sources and estimate the emission of the contaminant discharges into river networks. To evaluate the model, we used 227 measurements of the antibiotic Sulfamethoxazole with good results (R-squared = 0.65). The high resolution (500-meter) predictions of the model can be used to identify specific areas in the river network where high concentrations (or 'hot-spots') will tend to be found. The results can then be used as support for decision-making on focusing and prioritizing field studies, policy and management efforts, and technology resources.

4.03 Bayesian Networks: Applications for Environmental Risk Assessment and Management (Virtual Only)

4.03 Bayesian Networks: Applications for Environmental Risk Assessment and Management (Poster)

4.03.P-We172 A Bayesian Network Model of Mercury Pollution in Canada's Mackenzie River Basin

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High mercury concentrations in the Arctic Ocean are concerning for scientists and local communities. Methylmercury is a toxin that accumulates over time in living organisms, and exposure to mercury contaminated foods has been linked to developmental disorders in human fetuses. Fish consumption advisories impair Indigenous community access to affordable foods and infringe on inherent fishing and clean water rights. Mercury is a toxic heavy metal released through human activities such as fossil fuel burning and land disturbance, or through natural processes e.g., glacial melting releases mercury contaminants trapped in frozen soil and water. This study is the Canadian contribution to ARCRisk, a collaborative project between Canada, Russia, and Norway. The ARCRisk project aims to improve our understanding of the riverine contribution to Arctic mercury pollution. The Mackenzie River Basin was selected as the Canadian study site. The main objectives of the project are: 1) identify sources of mercury to land and water, 2) evaluate the associated risk for the environment and human health, and 3) develop an action plan to reduce environmental risk to the Arctic. To meet these objectives, I am developing a Bayesian-Network Relative Risk model (BN-RRM) capable of analyzing cause-and-effect relationships. BN-RRMs are used for risk assessment in large-scale systems and are depicted as webs connecting many stressors and outcomes. Bayesian Networks are visual representations of cause-and-effect relationships and facilitate communication of complex interactions to stakeholders. The model will identify risk factors and an action plan can be developed to target the influential source. This is an impactful and achievable master's project integrating existing datasets, human health assessment, traditional knowledge, and expert opinion.

4.03.P-We173 Bayesian Networks As a Tool for Predicting and Communicating Environmental Risks of Pharmaceuticals

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The ongoing COVID-19 pandemic has provided only the latest piece of evidence that communicating future risks to stakeholders and the public is a simultaneously vital and difficult task. Communicating the future risks of pollution in the environment builds on a broader, more nebulous, and less intuitive set of concepts, but is equally vital for safeguarding future human and environmental health. There will never be a single effective solution to a problem of this scale, but this by no means undermines the importance of developing tools that are simultaneously statistically rigorous and yet comprehensible to scientists, policymakers, and the public. We, then, present here our work on one item in the toolkit of risk characterisation and communication: a Bayesian network. Our more traditional approach to environmental risk assessment of pharmaceuticals is based on whole-sale data of pharmaceuticals in Norway for the years 2016-2019, which we have converted to predicted exposure concentrations for individual active pharmaceutical ingredients (APIs) (presented elsewhere). To make better use of this extensive dataset of exposure for environmental risk assessment, we are developing a prototype Bayesian network for the probabilistic calculation of risk quotients. The Bayesian network model will be used to assess the probability of exceeding regulatory threshold values (predicted no-effect concentrations - PNEC) for selected active APIs. The approach will be in accordance with the established process for the environmental risk assessment of pharmaceuticals in the EU, but with estimates of uncertainty retained throughout the process. Furthermore, we are expanding the model through the incorporation of predicted future conditions such as population growth and demographics, allowing for future environmental risk to be predicted under a variety of potential scenarios.

We are also developing a model version that will allow for the assessment of cumulative risk (probability of exceeding PNEC) for multiple pharmaceuticals with similar modes of action. With this Bayesian network, we hope to provide a case study in how Bayesian networks can be adapted to complex, real-world scenarios as a tool for prediction, communication, and explanation of environmental risks.

4.03.P-We174 Evaluating the Ecological Consequences of Altered Water Quality in South Africa Using Bayesian Networks *Victor Wepener¹ and Gordon O'Brien², (1)North-West University - School of Biological Sciences, South Africa, (2)University of Mpumalanga, South Africa*

Multiple water quality stressors derives from land use changes and formal anthropogenic sources including urban areas, agriculture, industries and mines have a legacy of impacting on receiving water resources. It is often very difficult to measure and predict the ecological consequences of these stressors on our socio-ecologically important rivers, lakes, wetlands and estuaries. It is also difficult to manage these stressors and as a result manage the sources from which they derive to achieve sustainable development. The management of water quality stressors and their sources are difficult because of the dynamism of the source-stressor-response relationships and multiple factors including lack of data, complex impact pathways and risks and uncertainties that are difficult to parameterise. Good scientific approaches that produce reliable impact projections of multiple water quality stressors are rarely applied to evaluate the ecological wellbeing of water resources, but Decision Analysis techniques commonly used in other fields have the potential to improve development decisions. This paper presents a Decision Analysis approach using a Bayesian Network (BN), risk based probability modelling approach to support decision makers manage multiple water quality stressors on multiple spatial scales. The BN probability modelling approach is widely applied in a range of disciplines, including medical sciences, genetics, environmental sciences and legal reasoning. It allows for the formal representation of causal models, such as risk probability modelling of multiple sources, stressors and receptors in the context of multiple ecological endpoints. This paper illustrates the use of BNs as a risk-based probability modelling approach to evaluate the probable ecological effects of altered water quality associated with multiple sources using the Vaal, Olifants, Inkomati, Thukela and uMvoti catchments in South Africa as examples. Available water quality, land use activity and ecotoxicology data were used to describe conceptual risk pathways, parameterise the BNs and model the probable consequences of multiple water quality stressors to water resources. Our models, based on available data, effectively represent the ecosystems considered in the case studies and using a scenario-based approach, predict the ecological consequences of altered water quality. This approach further identifies important, determining water quality constituents and associations to their sources so that the sources and their stressors can be managed to attain sustainable resource development.

4.03.P-We175 Bayesian Belief Networks As a Toolbox for the Creation of a Decision Support Framework for Plastic Clean-Up Technology Selection in Rivers and Estuaries

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With plastic debris accumulating in the marine environment at high rates, the need for sustainable mitigation strategies is imperative. Currently, one of the most used end-of-pipe solutions to block the transport of litter to the coast and ocean is the deployment of clean-up technologies in rivers and estuaries. These crucial technologies prevent plastic litter from reaching the marine environment, where it would rapidly spread and become more difficult to extract. However, to date, there is a lack of published empirical data on the potential impact of these devices on riverine and estuarine biota. Therefore, in this study, we propose to evaluate observation-based trade-off data for plastic clean-up devices to weigh bycatch versus plastic removal by using Bayesian Belief Networks (BBNs). Thus, creating a tool to support stakeholders in the decision-making process. We have identified four clusters of parameters that may play a substantial role in the bycatch of clean-up technologies, and that will be used to feed a BBN model as a starting point of a decision support framework. A first factor that will be considered in the BBN is the initial environmental status of the river or estuary in which the device will be deployed. In fact, transport of litter, including plastic debris, is highly influenced by the river's hydrological conditions. A typical example of this factor is the river flow velocity. A second factor to integrate into the probabilistic model is related to the traits of the biota present in the system and with which the device might interact. Size, buoyancy, and adhesiveness are all characteristics that might influence the chances of biota being unintentionally caught. A third important parameter is the characteristics of plastics present in the river or estuary such as size and buoyancy. Lastly, the removal operation mechanism of the clean-up devices is considered. To maintain the efficient collection of plastic debris while mitigating unintentional bycatch, we advocate that this BBNs model will support policymakers in the decision-making process, prior to the selection and deployment of plastic clean-up devices in rivers or estuaries. Policymakers will be able to find an optimal trade-off between plastic removal and possible negative environmental effects due to unintentional organic bycatch.

4.03 Bayesian Networks: Applications for Environmental Risk Assessment and Management (Poster Corner)

4.03.PC-We01 A Spatial Bayesian Belief Network for Assessing Field-Level Pesticide Pollution Risk in a Small Drinking Water Catchment

Mads Trolborg, Zisis Gagkas, Andy Vinten, Allan Lilly and Miriam Glendell, The James Hutton Institute, United Kingdom
Pesticides continue to present a significant risk to the quality of raw water for drinking water supplies worldwide. While catchment management is considered a cost-effective alternative to costly drinking water treatment, the effectiveness of pollution

mitigation measures is uncertain and needs to consider the complex interaction of local biophysical and agronomic factors. We present a spatial Bayesian Belief Network (BBN) that simulates inherent pesticide leaching risk to ground- and surface water quality to inform field-level pesticide mitigation strategies in a small drinking water catchment with limited observational data. The BBN accounts for the spatial heterogeneity in soil properties, topographic connectivity, and agronomic practices; temporal variation in climatic and hydrological processes as well as uncertainties related to pesticide properties and the effectiveness of management interventions. The BBN was used to simulate pesticide loss via overland flow and leaching to groundwater as well as the resulting risk of exceeding the regulatory threshold for drinking water for five different active ingredients. The effectiveness of mitigation measures such as delayed timing of pesticide application; reduction in application rates; field buffers; and presence/absence of soil pan on risk reduction were also evaluated. Sensitivity analysis identified timing of application, land use, presence of buffers, field slope and distance as the most important risk factors, alongside several additional influential variables. Pesticide pollution risk from surface water runoff was found to vary spatially across the study catchment, while groundwater leaching risk in general was uniform and low. Combined interventions of 50% reduction in pesticide application rate, management of plough pan, delayed application timing and field buffer installation was found to notably reduce the probability of high-risk from overland runoff and groundwater leaching. The advantage of the developed BBN is that it allowed to integrate diverse data sources in a dynamic field-scale assessment of 'critical source areas' of pesticide pollution in time and space, with explicit representation of uncertainties. The graphical nature of the BBN furthermore facilitated interactive model development and evaluation with stakeholders to build model credibility.

4.03.PC-We02 A Multiple Stressor Bayesian Network Ecological Risk Assessment for the Upper San Francisco Estuary
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The applications of the Bayesian network relative risk model (BN-RRM) recently have been recently reviewed. This report documents the application of these lessons learned to a risk assessment for the Upper San Francisco Estuary (USFE) in Northern California. The USFE is fed from a series of rivers that originate in the Sierra Nevada range and that form an extensive delta. Stressors include the inputs from agriculture, urbanization, industrial activity, and an extensive system of roads and other types of transportation. The region was divided into six risk regions capturing inputs from three major rivers, the different land uses, and urban areas. The Bayesian network identifies the inputs, the causal pathways, and the endpoints. The data are based on the SURF and CEDEN databases. We compiled an integrated database implementing extensive quality control measures to eliminate duplication, ensure the metadata, and organized by risk region. The models are built using Netica and followed the principles set forth in previous studies. Toxicity was documented using exposure-response curves as calculated using the drc package in R. Mixture toxicity was estimated using a novel additive curve fitting tool. The parameterized BN used to calculate the risk due to pesticides to overall fish mortality is an example of the overall process. The input concentration distributions for the pesticides were built using the observed amounts in that region. The concentrations for malathion, diazinon and chlorpyrifos are low but with long tails. Bifenthrin has a bimodal distribution with measurements at the higher concentrations. The risk calculation indicates that there is a 52 percent probability of the toxicity exceeding an EC₂₀ value in the Confluence risk region. We demonstrate that it is possible to generate a probabilistic Bayesian based risk assessment for multiple stressors to multiple endpoints even to a region as complex as the USFE. It is also possible to calculate the pesticide levels required to lower risk to acceptable levels. For the conference the interactions between the stressors and multiple other endpoints will be emphasized.

4.03.PC-We03 A Bayesian Network Tool for Predicting Fish Acute Toxicity Based on Fish Embryo Toxicity Test Data
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Fish Embryo Toxicity (FET) testing has been proposed as an alternative to using juvenile or adult fish in acute toxicity testing, to reduce the number of live animals required for hazard assessments of chemicals. The European Chemicals Agency has recommended the development of a Weight-of-Evidence (WoE) approach for strengthening the evidence from FET testing. While WoE approaches in the past have been largely qualitative, we have developed a Bayesian network (BN) model for using FET data in a quantitative and probabilistic WoE approach. The purpose of this BN model is to predict fish acute toxicity of a given chemical from data on fish embryo toxicity in combination with other types of available information (lines of evidence): quantitative structure-activity relationships (QSARs), toxicity to other species (algae and daphnids), and fish gill cytotoxicity. This presentation will focus on the final step of the WoE approach: the integration of the multiple LoEs and the methodology for weighting the LoEs. We will also present approaches to evaluating the model performance and defining its applicability domain. We consider the model presented here as a promising step towards a probabilistic WoE approach, with potential for further refinement and evaluation during the final phase of the SWiFT project. The next step is to finalise the web-based user interface to the model. A preliminary version of the online tool is available for testing purposes, and demonstrations of this tool can be provided upon demand. We consider the model presented here as a promising step towards a probabilistic WoE approach, with potential for further refinement and evaluation during the final phase of the project. While the purpose of this model is to predict acute fish toxicity from fish embryo toxicity, the approach can be relevant more generally for evaluating animal alternatives in regulatory toxicity testing, or even more generally for other types of environmental assessments.

4.03.PC-We04 Using a Bayesian Network Model to Predict Effects of Pesticides on Aquatic Community Endpoints in a Rice Field - a Southern European CASE Study

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Bayesian network models are becoming popular as a tool to support probabilistic environmental risk assessments. Compared to the deterministic approaches currently used in traditional risk assessment, Bayesian networks can better account for uncertainty. Our research focuses on the exposure of various pesticides in rice fields surrounding a Spanish Natural Park and their potential effect on the aquatic ecosystem. In this study, we use the Bayesian network as a meta-model that links the inputs and outputs for a process-based exposure model (RICEWQ) and a probabilistic case-based effect model (PERPEST). We run the exposure prediction model for various meteorological, hydrological and agricultural scenarios. The final developed Bayesian network enables the prediction of pesticide effects on several response classes of the taxonomic groups in an aquatic ecosystem. This approach also facilitates the communication of uncertainties associated with the risk calculation in a transparent way.

4.03.PC-We05 All That Glitters Is Not Gold - Should We Reconsider or Upgrade Bayesian Networks for Environmental Assessments?

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The many claims about the power and usefulness of Bayesian networks leave me with a spark of doubt – it sounds too good to be true! There are several things that make Bayesian networks fantastic. Bayesian networks are an icon of flexible modelling, allowing us to specify a probability model and integrate expert judgements with data, and query or update probabilities conditional on events. There are several challenges hampering the use of Bayesian networks for risk assessment. Bayesian networks can be used for different purposes and the models can be of different kinds. A Bayesian Belief network is a joint probability distribution over nodes representing states of the world or unique events, where the (subjective) probability distribution express uncertainty. A Bayesian network for multivariate data, can, e.g. as in machine learning, be trained to predict future data points or fill data gaps. Any risk assessment, concerned with variability in the system, as well as uncertainty about this variability, requires models able to separate these two things. Many applications of Bayesian networks mix variability and uncertainty (flat networks) or do not consider uncertainty (aleatory networks). This is partly driven by the availability of user-friendly Bayesian Belief network software, which has not been designed to make such a distinction. Bayesian models are agnostic to uncertainty and variability, and it is, therefore, a task for the assessors to attribute what parts of a model constitute variability, and make appropriate summaries to open up for communication of uncertainty in conclusions. Bayesian networks have gained a lot of attention as a promising method for risk assessment, but glorifying claims about these models are dangerous and may result in poor assessments. Instead, we must carefully reconsider what characteristics of Bayesian networks are desirable for each assessment, start with a clear conceptual understanding of the assessment model, use transparent and reproducible procedures to elicit expert judgments, integrate data and evidence, and consider uncertainties (including those outside the assessment model). Assessments need more use of complex statistical modelling relying on probabilistic graphical modelling and Bayesian inference. With some exceptions, it is only tradition that prevents the latter to be referred to as Bayesian networks. To ensure best practice in assessment, I recommend being precise about what we are modelling and upgrading our perception of what is a Bayesian network to include any probabilistic graphical model.

4.04 Contaminated sediment: an environmental compartment of concern

4.04.T-01 Recreation of Realistic Mixtures of Polycyclic Aromatic Hydrocarbons (PAHs) in Passive Dosing Bioassays

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Bioavailability is a key factor in ecotoxicological effects of contaminants and important for risk assessment approaches. However, total exhaustive extractions of contaminants do not consider the importance of the bioavailable fraction. Hydrophobic organic contaminants (HOCs), including polycyclic aromatic hydrocarbons (PAHs), are among the most common organic contaminants of concern. Due to their strong hydrophobicity, HOCs have their final destination in sediment, where their ecotoxicological effects are closely regulated by sorption and thus bioavailability. The North Sea is one of the most polluted marine regions in the world. The intensive use of this area leads to growing pollution of the ecosystem by chemicals. The mobility of HOCs, and consequently bioavailability, depends on their porewater concentrations. Currently, the freely dissolved concentration (C_{free}) is established as an important endpoint for sediment quality and risk assessment. In this study, solid-phase microextraction (SPME) was applied to examine the spatial and temporal distribution of C_{free} of PAHs in sediment cores sampled in the North Sea. The determined C_{free} were used to recreate realistic PAH mixtures found in North Sea sediments. These mixtures were then applied in miniaturized passive dosing bioassays to study their ecotoxicological effects on two species representing different trophic levels, namely *Raphidocelis subcapitata* as primary producer and *Daphnia magna* as primary consumer. Since nominal dosing often exhibits significant losses (e.g. due to sorption or volatilization), the passive dosing method was used to overcome these difficulties and to obtain stable exposure concentrations. The PAHs are released into the aqueous medium via passive diffusion from biocompatible polydimethylsiloxane (PDMS) O-rings, which serve as reservoirs for PAHs. The chemicals distribute between the silicone (C_{PDMS}) and medium (C_{free}) in the test vessels until thermodynamic equilibrium is reached. Bioassays with recreated PAH mixtures of North Sea pore water (C_{free}) were performed to assess their mixture toxicity. The field concentrations detected in the

sampled North Sea sediment cores inhibited the growth of *R. subcapitata* up to 95 %, whereas *D. magna* did not show a clear dose-response relationship when exposed to the same mixture concentrations. This suggests that *R. subcapitata* is the more sensitive species and that *D. magna* was not as susceptible to the exposure of realistic PAH mixtures.

4.04.T-02 Effect of Recent Mining Activities on Sediment Properties and Toxicity to Freshwater Organisms

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Active and closed mines are known to be a major threat to aquatic ecosystems, where metal inputs can result in decreased abundance and diversity of freshwater organisms. However, on a landscape level, metal exposure and environmental effects in mining impacted environments may vary over time and space. In addition, factors affecting the bioavailability and toxicity of metals, such as total organic carbon (TOC), may also vary over time due to mining activities. These temporal and spatial patterns are important to understand in order to assess the status, the potential recovery and the need for post-treatment in mining impacted environments. In this project we used the geochemical record preserved in lake sediment together with estimates of toxicity for aquatic organisms to examine the long-lasting effects of past, century-scale mining. We used sediment cores from three small lakes affected directly or indirectly by mine tailings from an abandoned mine. The lakes that had served as clearing ponds were more severely affected than the lake receiving indirect discharge from tailings. However, all lakes showed clear impact from the mine, with reduced TOC and increased input of metals (Pb and Zn) at levels that most probable caused negative biological effects. The peak in Pb accumulation differed in time, depending on when and how long the clearing ponds had been in use. Probable Effect Concentration Quotients (PEC-Q) indicated that although the risk for toxic effects became much reduced after mining ceased, there is still a high risk for biological effects in the old clearing ponds. This was also evident after PEC-Q were TOC-normalized. However, in the lake receiving no direct discharge from mine tailings, a biological recovery is expected within the near future. We conclude that even within the same catchment, the effect and recovery in metal contaminated lakes may differ and we further suggest that TOC-normalized PEC-Q, rather than PEC-Q should be used to assess the toxic risk of metal contaminated sediments.

4.04.T-03 Wastewater Treatment Plant Contaminant Profiles Affect Macroinvertebrate Sludge Degradation

Tom Vincent van der Meer¹, Piet Verdonschof², Lisa van Eck³, Dominique Mon-Coeur Narain-Ford³ and Michiel Kraak⁴, (1)Wageningen Environmental Research - WUR, Wageningen, Netherlands, (2)Wageningen Environmental Research, Netherlands, (3)Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Netherlands, (4)University of Amsterdam, Netherlands

Disposal of excess sludge produced by wastewater treatment plants (WWTP) can make up 60% of the operational costs. Methods that may reduce the amount of excess sludge are therefore urgently required. As macroinvertebrate detritivores play an important role in the degradation of organic matter in the aquatic environment, they may also be capable to degrade WWTP sludge. Yet, interactions between detritivores as well as high contaminant concentrations in the sludge may affect the sludge degradation rate by macroinvertebrates. The aim of the present study was therefore to examine the degradation of sludge from WWTPs differing in contaminant profiles by three benthic macroinvertebrates, individually and in all possible species combinations, where we tried to link the observed macroinvertebrate sludge degradation to the WWTP specific contaminant profiles. Subsequently, we experimentally assessed the effect of contaminants on sludge degradation and biotic interactions, by spiking WWTP sludge with Cu. Our results showed that most macroinvertebrate (combination) treatments enhanced sludge degradation in a species and WWTP specific way. Positive biotic interactions were only observed at high contaminant loads, where sludge degradation rates were reduced. In the Cu spiked sludge, degradation decreased with increasing Cu concentrations, but the sludge degradation in the species combination treatment was higher than expected based on the degradation by the single species. Even though WWTPs differ in their contaminant profile and load, employing (combinations of) detritivorous macroinvertebrates offers a promising approach to reduce the amount of excess WWTP sludge, where the feasibility will depend on the possibilities of upscaling these methods.

4.04.T-04 Biotransformation of Benthic Invertebrates Impact Persistence and Bioaccumulation of Sediment-Associated Cyclic Volatile Methyl Siloxanes (D4, D5, D6)

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Regulatory frameworks generally assess risk of hydrophobic organic chemicals (HOCs) based on their persistence, bioaccumulation and toxicity using water-exposure setups and microbial degradation. However, this may misjudge the risk of HOCs, such as cyclic volatile methyl siloxanes (cVMS), due to their accumulation in the sediment compartment. We assessed the impact of dietary uptake (i.e., via sediment ingestion) and benthic invertebrate biotransformation for risk assessment of cVMSs in a number of studies. A radiotracer technique was employed to examine uptake- and depuration kinetics as well as biotransformation of sediment-associated cVMS (octamethylcyclotetrasiloxane, D4; decamethylcyclopentasiloxane, D5; dodecamethylcyclohexasiloxane, D6) by a freshwater oligochaete (*Tubifex tubifex*) and a marine polychaete (*Capitella teleta*). Overall, sediment-associated D4, D5 or D6 did not adversely affect the benthic invertebrates. Uptake, depuration and biotransformation was dependent on compound. BSAF was low due to biotransformation and fast elimination of parent and metabolites. Worm presence reduced sediment cVMSs (up to 95% for D4), especially in the estuarine system inhabited by *C. teleta*, to levels exceeding microbial degradation alone. It, thus, appears that cVMSs have little impact on sediment-dwelling

organisms at environmentally realistic concentrations. Our results highlight the importance of benthic invertebrate biotransformation in reducing uncertainty in B and P assessment of HOCs, like cVMSs.

4.04 Contaminated sediment: an environmental compartment of concern (Poster)

4.04.P-Th123 Assessing Sediment Status in Europe: Frameworks, Standards and Approaches, an Expanded Review

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It is nearly two decades after passing the Water Framework Directive, which mandated catchment-wide approaches to managing waterways, and over a decade since the European Sediment Research Network (SedNet) published a series of books addressing the role of sediments in achieving this goal. In 2008, the European Commission published a directive on environmental quality standards in the field of water quality, but declined to specify or require sediment quality guidelines except under special circumstances, although it still required that Member States monitor sediment and biota for priority substances that accumulate in them. Subsequently, a significant body of work has addressed contaminant trends, drivers of toxicity and contaminants of emerging concern in European waters. While European approaches to sediment assessment and monitoring were reviewed by the SedNet, many national approaches have evolved since that time, or are under review. Documents on national approaches to freshwater and marine sediment assessment were reviewed for a range of European countries. An additional focus was on evaluating both national approaches to emerging contaminants not currently identified as priority pollutants, and how future attention on these might change sediment assessment frameworks in the near term. As far as was possible, national approaches were summarized in a common context. **1. 2.** Europe remains quite diverse in its approach to sediment standards and assessment. Approaches range from a complete absence of standards and guidelines to highly detailed frameworks; applications can be broad or narrow. The assumptions behind frameworks and policies are critical to their application; not all countries clearly distinguish between screening and action levels. Where specified, lists of potential contaminants to be monitored range from a handful to dozens, and differ for freshwater and marine systems. This paper will update a 2017 review and compare approaches to sediment chemical assessment in a much broader range of European countries and regions. Trends and changes, and the relationship between chemical and other assessments, will be discussed.

4.04.P-Th124 Investigations on the Risk Assessment of Soft Plastic Baits - Determination of the Content of Phthalic Acid Esters and Polycyclic Aromatic Hydrocarbons in Fishing Lures and Their Delivery to the Water Phase

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The offer of soft plastic baits (SPL) is large in the fishing stores. These baits are mostly made of soft PVC, which can contain about 70% plasticizer (i.a. phthalic acid esters) and also polycyclic aromatic hydrocarbons (PAH) as contaminants. These chemicals can be endocrine disruptors (phthalates) and toxic (PAHs). Lures are often lost when they get caught on the ground or torn off by the fish. But how many get lost and do they harm? The German city of Hamburg is a case in point. About 120,000 anglers are registered here. This high number of anglers meets only a small water area. Therefore, it is obvious that the load of torn baits can be locally high. To assess the risk of SPLs, the study investigated which plasticizers and contaminants are present in selected SPLs and how they enter the water phase. The analytes were extracted from the baits using solvents and measured by GC-MS technique. Selected baits were placed in water for several weeks. Water samples were extracted and measured regularly. It has been observed that some SPLs release attractants into the water. These consist of organic material and may have an influence on the release of pollutants. The most commonly detected phthalates were Diisononyl phthalate (DINP) and Diisodecyl phthalate (DIDP). The phthalates Dibutyl phthalate (DBP) and Benzyl butyl phthalate (BBP) were not found. The substance Di(2-ethylhexyl) phthalate (DEHP) was detected as an individual case in a single sample. PAHs were identified in some examined artificial baits. The concentrations of phthalates and PAHs are partly above the limits set on toys for young children. A risk for children can therefore not be excluded. For some phthalates and PAHs, a significant increase in concentration was observed in the water phase. In addition, a continuously increasing turbidity intensity was observed emanating from the attractants. The achieved concentrations of some phthalates clearly exceed the theoretical solubility. It can be discussed that the attractant release is a driving factor for the pollutant release. As a result, significantly more pollutants are released at higher rates. Thus, the use of SPLs increases the local risk of the pollutant release into the water. Therefore, it would be desirable to use either alternative degradable materials or alternative techniques. The awareness of the harmfulness of PVC baits is the driving force for this. It can be conveyed, for example, through the fishing license examination.

4.04.P-Th125 Derivation of Environmental Quality Standards for Sediments: Swiss Experience With Traditional and Emerging Sediment Contaminants

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The Swiss Centre for Applied Ecotoxicology (Ecotox Centre) has derived environmental quality criteria for surface waters for many substances, including pesticides, biocides, pharmaceuticals and industrial chemicals [1]. The derivation is largely based on the EU Technical Guidance for Deriving EQS (TGD for EQS; [2]), because the protection goals of the Swiss water protection law and ordinance and the Water Framework Directive (WFD) are comparable. In contrast to the WFD dossiers, sediment and human health via the consumption of fisheries products are not part of the EQS derivation in Switzerland. In the last years, the Ecotox Centre has been working on a sediment quality assessment strategy for Switzerland [3]. The objective is to harmonize and improve practices in sediment quality monitoring and assessment in Switzerland, which mainly addresses the chemical status so

far. The strategy proposes procedures for sediment sampling and analysis and a list of 20 substances for which information on measured environmental concentrations in sediments should be gathered to assess their occurrence at the national level. In this context, sediment EQS were derived. Here we report on the experience with deriving sediment EQS for 20 substances that include both, traditional sediment contaminants such as metals, PAHs or PCBs, and contaminants of emerging concern such as pesticides, pharmaceuticals or personal care product ingredients. We will present the derived values for each substance but will mainly focus on providing feedback from the derivation process, in particular on the availability of relevant ecotoxicological data and the quality and reliability of available data. We will also discuss the performance and potential issues with implementation of the derived EQS for sediments.. [1] Korkaric M, Junghans M, Pasanen-Kase R, Werner I. 2019. Revising environmental quality standards: lessons learned. *Integr Environ Assess Manag* 15: 948-960. [2] European Commission. 2011. Technical guidance for deriving environmental quality standards. Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance document No. 27. Prepared by EU, member states and stakeholders. Technical Report?2011?055. [3] Casado, C., Wildi, M., Ferrari, Benoit J.D., Werner, I. 2021. Strategie zur Beurteilung der Sedimentqualität in der Schweiz. Studie im Auftrag des Bundesamts für Umwelt. Schweizerisches Zentrum für angewandte Ökotoxikologie, Lausanne.

4.04.P-Th126 Retrospective Screening Analysis for Biocides in Suspended Matter at Urban Locations

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Biocidal substances enter the aquatic environment through numerous entry pathways. One main entry path is through the sewer system. Biocides entering the sewage treatment plants (STP) are often incompletely removed and thus the parent compounds as well as their transformation products are discharged into the water body. Moreover, a direct discharge of biocides into surface water can occur via rainwater discharges in separate sewers and in case of combined sewers also via the combined sewer overflow (CSO) during heavy rain events. In a recently finalized project, 29 STPs distributed all over Germany were investigated for selected biocides. It was shown that about 75 % of the 26 analyzed substances pass the STP. The results also confirmed relevant biocide concentrations in CSO released into the surface water. Due to their adsorption properties, it can be assumed that some of these biocides will also accumulate especially in suspended matter. Suspended solids are important structural and functional elements in aquatic ecosystems and also a suitable matrix for retrospective monitoring in order to assess temporal and regional trends. However, for many biocides data on concentrations in suspended matter are still scarce. Thus, the aim of the presented project is to expand the knowledge on biocides in suspended matter. For this purpose, 1) a retrospective quantitative analysis for selected biocides (e.g. quaternary ammonium compounds (QACs), azole fungicides, pyrethroids) is carried out at different urban locations over a period of 7-8 years and 2) a semi-quantitative analysis of further biocides via suspect screening is conducted. By focusing on urban locations, relevant biocides and their entry path should be traced. For this purpose, the new data are linked with existing findings of biocides in STP effluents in Germany. The extension of the monitoring to the particulate phase enables the detection of (strongly) sorptive substances even in low concentrations and thus a better knowledge of the distribution of these substances in surface waters. Finally, the results provide a time-dependent picture of the environmental pollution by biocides in Germany and also show possible fields of action for regulatory purposes. First results available so far confirm the wide spread occurrence of biocides in particular matter. Especially QACs such as Dimethyldidecylammonium were found to be ubiquitously present in high concentrations of up to 6 µg/g dry weight.

4.04.P-Th127 Uptake and Accumulation of Amitriptyline in the Polychaete, *Nereis Virens*, Exposed to Field-Collected Sediment Near a Wastewater Treatment Discharge Site in Norway

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An increase in the global consumption of pharmaceuticals and abundance of residues detected in the environment has raised concern to the health of aquatic organisms. Wastewater treatment plants (WWTPs) discharge represents a major source of pharmaceuticals to the aquatic environment due to an incomplete removal of compounds prior to release in freshwater and marine systems. Following sampling events in the surroundings of a WWTP outfall in Stavanger (Norway) in 2019 and 2020, sediment samples collected were comprised of several representative classes of pharmaceuticals. One compound in particular, a tricyclic antidepressant, amitriptyline, was detected in sediment samples and of particular interest as there is relatively little known regarding its capacity to accumulate in benthic organisms from contaminated sediment. To better understand the potential impact of amitriptyline in the marine environment and its ability to accumulate in biota, marine benthic polychaetes, *Nereis virens*, were exposed to field-collected sediment samples from Kvitsøy (reference), Boknafjord, and WWTP outfall, as well as 3.06 µg/g and 30.57 µg/g amitriptyline-spiked sediments for 28-days. The average amitriptyline concentration detected from sediment exposure samples were comprised of the following: Kvitsøy (12.7 ± 8.0 ng/g), Boknafjord (15.6 ± 12.7 ng/g), WWTP outfall (6.5 ± 3.9 ng/g), 3.06 µg/g amitriptyline-spiked sediment (423.83 ± 33.1 ng/g), and 30.57 µg/g amitriptyline-spiked sediment (763.2 ± 180.5 ng/g). *N. virens* had body burden concentrations of amitriptyline below the limit of detection (LOD; 3.5 mg/mL) following exposure to Kvitsøy and WWTP outfall sediments, whereas detectible concentrations were measured from Boknafjord (5.2 ± 2.8 ng/g) and those exposed to 3.06 µg/g (9.5 ± 0.2 ng/g) and 30.57 µg/g amitriptyline-spiked sediments (56.6 ± 2.2 ng/g). There was a positive correlation between the concentration of amitriptyline in sediment samples and body burden concentrations in *N. virens*, which suggests that amitriptyline has the potential for trophic transfer following exposure to contaminated sediments.

4.04.P-Th128 Toxicity Assessment of Dredged Sediments From the Lagoon of Venice Using the Clam *Ruditapes philippinarum*

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The Lagoon of Venice is an important and complex coastal ecosystem of the Northern Adriatic Sea, the safeguard of which is fundamental for biodiversity protection and ecosystem services. Human activities caused the direct and indirect increase of inorganic and organic pollutants in seawater. The Lagoon of Venice often undergoes dredging of canals to allow the transit of ships and sediments can be dumped at other sites with unknown effects. Resuspension of sediments can allow toxicant release, making them available to filter-feeders, such as clams, with potential risk for consumers. Here, the toxicity of sediments from six sites of the Lagoon of Venice was assessed on the Manila clam, *Ruditapes philippinarum*. Five sampling sites were located between the city center of Venice and the industrial area of Porto Marghera. The sixth sampling site was selected far from the others, in an area with presumably low levels of contamination. Four sediment cores were collected at each sampling site. The clams were experimentally exposed to the sediments for 3 and 14 days and a battery of biomarkers was measured. The capability of sediments to induce oxidative stress and damage, as well as neurotoxicity, and to alter detoxification properties of clams was assessed both in gills and the digestive gland. The experiment was repeated in two seasons (winter and spring), in order to highlight a time-dependent toxicity of sediments and to reveal a different sensitivity of clams related to different periods of their biological cycle. For most of the biomarkers investigated there was a significant effect of the variables “exposure time”, “sampling site” and their interaction. The results revealed differences in the responses of the clams between the two experiments, with a possible influence of the clams’ reproductive phase. In general, a clear pattern could not be identified, and clams from the sixth site showed similar responses to those of bivalves from the other sites. Oxidative damage in clam tissues was low, suggesting the ability of the animals to cope with contaminants. A different pattern of contamination among sites is suspected. However, chemical analyses of sediment samples are still ongoing. Scientific activity performed in the Research Programme Venezia2021, coordinated by CORILA, with the contribution of the Provveditorato for the Public Works of Veneto, Trentino Alto Adige and Friuli Venezia Giulia.

4.04.P-Th129 Multidisciplinary Assessment of Estuarine Sediments Collected in the Bay of Biscay

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In recent decades, pollution in estuaries has been one of the major environmental problems that affected waters but also sediments and organisms inhabiting them. Estuarine sediments accumulate high levels of discharged wastes, acting as a sink and source of pollution. Metals are the most studied and monitored pollutants in sediments chemical characterization. However, in order to perform a multidisciplinary assessment of polluted sediments biological effects in environmentally relevant species should be considered. The polychaete, *Hediste diversicolor*, was chosen as test organism since it is an endobenthic species, widespread in sediments. With this species, innovative *in vivo* and *in vitro* tests were performed to assess the toxicity of sediments collected in 4 estuaries of the Bay of Biscay. Sediments with different pollution inputs (present and past) were sampled in the Nerbioi-Ibaizabal (Udondo, Kadagua, Benedicta, Zorroza), Butroe (Plentzia-reference-), La Gironde (Plassac) and Charente (Rochefort) estuaries in October 2018 and March, July and October 2019. Physico-chemical characterization was done and the mERMq cumulative index was calculated according to the metal content. For *in vivo* assays, adult polychaetes were introduced into the sediments and after 7 d of exposure, metal accumulation, mortality, weight loss, histopathology and biochemical responses were measured and integrated in a Biological Response Index (IBR). Regarding *in vitro* approaches, immune cells (coelomocytes) were extruded from the coelom, exposed to sediment elutriates (DIN 38414-S4) and after 24 h their viability was recorded (Calcein-AM viability assay). In parallel the Sea Urchin Embryo Test with estuarine sediment elutriates was carried out (ICES, 2012). The characterization indicated that sediments collected at different estuaries and seasons were all loamy and high in OM (>3%), with the exception of Benedicta. mERMq and IBR values indicated that sediments from Udondo and Benedicta had the highest toxicity with a concomitant decrease in coelomocytes viability, lower growing capacity of sea urchin larvae and embryo malformations. The sediment toxicity bioassays implemented herein could be helpful for risk assessment, to define Sediment Quality Guidelines and for dredging operations management. *Acknowledgement*: Basque Gov. (IT1302-19, IT1213-19), U. Basque Country and MINECO (CTM2017-87766-R).

4.04.P-Th130 Multiple Stress Factors Affecting Sediments in the Estuaries of Elbe and Odra

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Estuaries are vulnerable to multiple human impacts threatening the long-term viability, ecological function and health of these important ecotones. Pollutant and nutrient inputs, increasing industrial pressure, habitat loss and climate change represent the most important stressors in estuaries. In this diverse range of pressures, scientifically sound sustainable management is a challenge. The preservation of healthy and viable estuaries therefore requires a detailed understanding of the essential characteristics and linkages in the estuarine systems, as well as novel measures to preserve these valuable coastal systems. The Blue Estuaries project (BluEs) aims to improve the understanding of the complex estuaries Elbe and Odra with regard to the interaction of different anthropogenic and natural process (e.g. hypoxia, eutrophication and chemical pollution) on the basis of a comparative analysis. One of the main objectives of our investigations within BluEs is the role of sediment bound contaminants in the Elbe and Odra estuaries. For this purpose, sediment and water samples will be collected over the course of 2 years along stress gradients. Their

ecotoxicological effects on organisms of different trophic levels (e.g. bacteria, microalgae and nematodes) will be investigated by applying various biotests such as bacterial contact test, algae growth inhibition test and the acute daphnia test. By analysing the toxic endpoints, undesirable effects of single and combined stressors on the biological community will be detected. The effects of new substances with specific modes of action, such as endocrine disruptors or herbicides, will be measured using reporter cell lines and pulse amplitude modulation (PAM) fluorometry. The concentrations of pollutants in the sediment and water samples will be analysed, and the Toxic Unit concept will be used to determine how much of the ecotoxicological effect can be attributed to the measured pollutants. The chemical and ecotoxicological data will be compared with the meiobenthic community data (Nema-SpeAR-Index) to assess the quality of the local biological communities. All results and relevant data collected in the project will be compiled and analysed, in form of a qualitative/quantitative model of complex cause-effect relationships, which will help to improve the understanding of the complex interactions in the estuaries. This poster will introduce the project and its first results.

4.04.P-Th131 Exploring Chemical and Biological Data to Understand the Impacts of the Pollutants in the Doce River, Brazil, After the Mining Dam Collapse

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High levels of hazardous chemicals may be introduced into aquatic ecosystems and concentrate in bottom sediments during episodes of acute and chronic environmental contamination. The Fundão Dam failure in 2015 represented one of the worst environmental disasters in Brazil, discharging more than 43 million of m³ of iron-rich tailings wastes into the Doce River Basin (DRB), resuspending the chemicals deposited in the sediment and causing irreversible socio-environmental impacts. Although many studies initially reported the anomalous levels of metals in water, the longer-term impacts considering the influence of environmental factors and other pollution sources are yet to be determined, as well as potential effects on the exposed organisms. This study investigated the impacts of the pollutants from the Doce River four years after the mining dam collapse, considering the analysis of inorganic and organic chemicals in water and sediment, associated with biomarkers responses of wild fish and fish embryos exposed to elutriate solutions. The diagnostic indexes and multivariate analysis revealed that sampling sites at the Upper DRB, closest to the mining activities, showed the worst sediment quality, with the highest levels of trace elements, including the hazardous As and Hg, which exceeded the federal standards (CONAMA). Major stress responses were observed for fish from these sites, such as higher lipid peroxidation and metallothionein expression, and depleted GSH, as well as higher deformities rates of fish embryos. On the other hand, concentrations of PAHs in the sediments were higher in the Middle and Lower regions of the DRB. Benzo[a]anthracene exceeded legal limits, and fish exhibited higher DNA damage and increased AChE activity. In addition, PCA analysis showed the seasonal variation as an important factor influencing the distribution of chemicals, as the rainy season increases the levels of trace elements in water due to the higher resuspension of sediment. The integration of chemical analysis with biological responses allowed us to better understand the environmental impacts across the DRB, as a result of the historical ore exploration, combined with the dam failure disaster, which spread the inorganic contaminants along the DRB, and other contamination sources, such as those responsible of releasing organic chemicals. These sources have been historically neglected in the DRB, but their contributions, when summed to those from mining, result in complex mixtures of pollutants, increasing the ecological risks in the DRB.

4.04.P-Th132 Ecotoxicological Evaluation of a Floodplain Restoration Project Near an Industrial Plant

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Floodplains are among the most species-rich ecosystems in Central Europe and are therefore of great importance for biodiversity conservation. In addition, they fulfill a variety of ecological services, such as water and air filtration, carbon fixation, or urban microclimate improvement. Over the past decades, however, rivers have been channelized and structurally degraded to allow navigation, gain land and protect against flooding. The European Water Framework Directive (EU-WFD) demands the implementation of several floodplain protection measures. Accordingly, the City of Frankfurt am Main (Hesse, Germany) has initiated the restoration of a 90 ha floodplain, which to date is the most extensive renaturation measure on the Hessian section of the river Main. The planning includes two tributaries and several oxbow ponds, bank flattening, meadows, and floodplain forests. Some of these structures have already been established between 2014 and 2019. About 1/3 of the area remains available for conventional agriculture. The project is particularly interesting from an ecotoxicological perspective because the plan area is located next to an industrial plant that discharges treated wastewater 1.5 km upstream of the inflow of the future tributary. Regarding the major flood events of the last decades, pollutants from the Main river and the effluents of the industrial plant, which had no wastewater treatment plant from 1870 to 1981, are hypothesized to have been deposited on the floodplain. Previous studies have shown that, in addition to hydromorphological restoration measures, good water and sediment quality is crucial to improve the ecological status of water bodies according to the EU-WFD. In the project, water and sediment samples from the Main river and the existing water bodies in the floodplain will be analysed, as well as soil samples from the future tributary. For an ecotoxicological evaluation of the restoration, the samples are tested for baseline toxicity (Microtox assay), mutagenicity (Ames

fluctuation test), endocrine and dioxin-like activities (yeast reporter gene assays, ?EROD- and CALUX® assay), neurotoxicity (AchE inhibition test), oxidative stress response (AREc32 assay) and *in vivo* fish embryo toxicity (FET). Additionally, a selection of samples will be chemically analysed for organic pollutants by the Helmholtz Centre for Environmental Research using liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS).

4.04.P-Th133 Combined Chemical and Bioassay Analysis of Polycyclic Aromatic Hydrocarbons (PACs) in Sediment From the Coast of Gulf of Bothnia

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The marine environment is a sink for polycyclic aromatic compounds (PACs) where their occurrence and levels are strongly affected by current and historic anthropogenic activities. Though the diversity of PAC spans over a multitude of different classes and compounds, only a limited number of PACs is usually assessed in current regulatory frameworks of sediments. Recent years, attention has increased towards PACs other than the priority PAH16, such as alkylated PAHs, heterocyclic PACs, and oxy-PAHs, due to their toxicity and environmental persistence. In this study, a wide range of PACs were analyzed in sediment samples from the coast of Gulf of Bothnia; PAHs, alkylated PAHs, oxy-PAHs, O-PACs, N-PACs, and S-PACs, in total 85 compounds.

Bioanalytical analysis was performed with three cell-based assays targeting aryl hydrocarbon receptor (AhR) activity, estrogenic activity and antagonistic androgenic activity; PAH CALUX, ER CALUX and anti-AR CALUX. Chemically derived benzo[a]pyrene (BaP) equivalents (chem-TEQs) were calculated from the relative response potency factors (REPs) specific to the PAH CALUX and compared to the derived bio-TEQ values. Extraction was performed with selective pressurized liquid extraction (SPLE) using basic silica and dichloromethane. The method was validated with spike and recovery experiment using low-contaminated sediment and showed good performance for all included analytes. The results showed ?PAC concentrations ranging from 2.8 – 12 ng/g d.w. Up to 67 PACs were found in the sediment samples. Comparison of the bioanalysis and the target PAC analysis revealed that chem-TEQ values of PAH16 could explain 12 – 84%, on average 34%, of the bioactivity in the PAH CALUX. Only a few samples showed significant activity in the ER CALUX and anti-AR CALUX.

4.04.P-Th134 Biotransformation of Cyclic Methyl Siloxanes by Benthic Invertebrates - a Proof of Concept Approach

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Hydrophobic organic contaminants (HOCs) will inevitably end up in the aquatic environment where they will settle out and accumulate in the sediment. The environmental fate of HOCs depends on several factors, including microbial degradation but also biotransformation by benthic macrofauna. Macrofaunal biotransformation have been reported to impact the persistence of sediment - associated HOCs to a higher extend than microbial degradation. Among the HOCs, cyclic volatile methyl siloxanes (cVMS) have a high hydrophobicity ($\log K_{ow} > 5$) and are defined as “superhydrophobic” (i.e., $\log K_{ow}$ approach or exceed 7) rendering them to bind particles in the water column and eventually accumulate in sediments to levels much higher than in the overlying water. The half-life of the most common cVMS in sediment is much longer than in water: D4 half-life is 3.9 days in water and 365 days in sediment; D5 half-life is 70.4 days in water and 3100 days in sediment. However, results from our laboratory demonstrate much lower half-lives in sediment inhabited by the estuarine polychaete, *Capitella teleta*, and the freshwater oligochaete, *Tubifex tubifex* due to a high biotransformation capacity. Deposit-feeding invertebrates, such as *C. teleta* and *T. tubifex*, colonize and thrive in organically polluted areas where they often reach high densities (e.g., >100.000 pr m^2). A high biotransformation capacity, as shown for several Persistent Organic Pollutants, may explain their ability to live in organically polluted areas. However, information on the mechanisms involved in biotransformation processes are limited. Our hypotheses are that *C. teleta* and *T. tubifex* biotransform cVMS through the induction of CYP450 enzymes and/or have a unique microbiome that metabolize the contaminants to more water-soluble compounds. In addition, methods to assess biotransformation of compounds with unknown metabolites is lacking. The aim of this study is to examine the mechanisms behind cVMSs biotransformation in *C. teleta* and *T. tubifex*. Here we suggest a conceptual approach to assess the biotransformation potential in benthic invertebrates using cVMS as model compounds. *Acknowledgement* - The authors acknowledge Centre European des Silicones (CES, Project no: 021444/5040/07/0226) for its financial support.

4.04.P-Th135 Organophosphate Esters Interactions With Microbial Assemblages in Coastal Sediments Under High Urban Pressure

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The widely used and hazardous organophosphate esters (OPEs) flame retardants and plasticizers can effectively accumulate in marine sediments, and coastal sites may represent in some cases important sedimentary OPE stocks ^{[1][2]}. Open questions at present are on their field persistence and the capability of naturally occurring microbial communities in “contaminated sediments” from marine coastal areas to effectively degrade OPEs, enhancing their in-situ degradation, and on their potential impacts of these plastic additives on these communities. To address this issue, we applied an integrated chemical-microbiological approach on a sediment in the impacted area in the vicinity of the Marseilles’ WWTP outlet (NW Mediterranean) receiving constant OPE inputs. Immediately after sediment collection and homogenization, thirty-nine sub-samples (40 g of wet sediment) were spiked with an OPE mix to achieve environmental relevant concentrations and incubated in dark using a temperature-controlled chamber ($13 \pm 1^\circ\text{C}$) during one month. Two conditions (*i.e.* abiotic, biotic) were tested and samples were analyzed at T0 and after 7, 14 and 30 days corresponding to T1, T2 and T3, respectively. OPE were quantified by GC/MS after ultrasound extraction and clean-up, and

total sedimentary DNA was extracted in each treatment to quantify the number of bacterial 16S rRNA genes (proxy of bacterial abundance) and to characterize the structure and composition of the bacterial community (16S metabarcoding MiSeq Illumina) throughout the experiment. The degradation of seven OPEs, including non-chlorinated and chlorinated compounds was studied. Overall, OPE degradation proceeds faster under biotic conditions. For example, the $t_{1/2}$ for two alkyl-OPEs: the low molecular weight tri-n-butyl phosphate (TnBP) and the high molecular weight tris(2-ethylhexyl) phosphate (TEHP) were of 46 and 77 days (abiotic conditions) compared to 19 and 47 days in biotic conditions, respectively. However, chlorinated OPEs exhibited similar degradation rates under both conditions. The bacterial community of the Marseilles' WWTP outlet sediments are dominated by 4 bacterial phyla (*Proteobacteria*, *Bacteroidetes*, *Calditrichaeota* and *Epsilonbacteraeota*). OPEs didn't induce a significant effect on the structure of the prokaryotic community nor on the alpha diversity. There was however a stimulation of the growth of the bacterial community in the first days after the spiking with OPEs. Data treatment is still undergoing, and these results will be valuable data for the refinement of OPE chemical risks assessment methodologies

4.04.P-Th136 Structural Changes in Chironomus riparius Exposed to Iron Oxide Nanoparticles

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In the last two decades, a large amount of data has emerged on the potential effects of factory-produced and processed nanomaterials on organisms living in freshwater, soil and sediments. The Organisation for Economic Co-operation and Development (OECD) and Widening Participation Medics Network (WPMN) point out that existing toxicological protocols can be used to test the toxicity of nanoparticles on chironomids, but with special reference to parameters that could affect the toxicity of the tested nanoparticles and the interpretation of the results. Through this research, the sensitivity and practicality of Geometric morphometry method for assessing the impact of iron oxide nanoparticles (nano-Fe₃O₄) was examined. Mouthparts of larvae (mentums and mandibles) and forewings of adults were analysed to determine all discrete differences in the size and shape of structures and associate the results with different nanoparticle concentrations in sediments. This method revealed differences in the structures of treated and untreated individuals, although no effect on standard life parameters was shown. Improving the testing methodology using chironomids as model organisms, can greatly improve the further monitoring of the state of aquatic ecosystems and the biomonitoring of nanoparticle pollution, and thus the possibility of early purification and remediation.

4.04.P-Th137 Testing Toxicological Sensitivity of Cultured Vs Newly Sampled Mud Shrimps, Corophium volutator

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When assessing the ecological effects of contaminants in the environment, well-studied test-organisms are a crucial tool when performing standard-tests (e.g. OECD guidelines). When conducting standard water-only tests, organisms used are in general well tested and documented (e.g. *Daphnia magna*), however, when assessing toxicity of sediment systems, organisms are in general less well described. OECD have previously suggested and many have used the mud shrimp, *Corophium volutator*, as a tests organism when assessing toxicity of estuarine- or marine sediment systems, based on *C. volutator* being a cosmopolitan species, thriving in a broad range of salinities (from fully saline to almost freshwater), having high sensitivity, and constitute a substantial part of the diet of many fish and shore birds. However, field collected specimens are often used, as laboratory cultivation of *C. volutator* may be challenging and as amphipods in general may decrease in sensitivity already after 15 days in culture. The aim of this study was to test how culture time (< 15 days, 1 month, >1 year) impact sensitivity of *C. volutator*. The mud shrimps were exposed to cadmium chloride (CdCl₂) in a concentration-response water-only setup to nominal concentrations of 1, 3, 7, 10 and 15 mg L⁻¹ CdCl₂ and a control assessing mortality after 72 and 96h. In general, organisms cultivated for < 15 days seemed to be more sensitive towards CdCl₂ after 72h of CdCl₂ exposure. However, after 96h exposure to CdCl₂ the differences between the groups was almost diminished. The results further suggests that it is merely when performing short-term exposures (less than 4 days), where it is important to use field-collected *C. volutator*. We suggest to use cultured *C. volutator* as test-species for toxicological testing in sediment systems for experimental time frames longer than 4 days, e.g. 10 day acute sediment tests. Lastly, would we like to emphasise the need for further studies to investigate the most optimal conditions for keeping *C. volutator* in culture.

4.04.P-Th138 Moving Towards Chemical Activity-Based Sediment Toxicity Tests

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The chemical activity or effective concentration of hydrophobic organic contaminants (HOCs) can provide a highly relevant metric for the risk assessment of polluted sediments. Many HOCs can exert baseline toxicity to benthic organisms over a narrow but consistent range of activities. As chemical activities are additive, the approach works for both single compounds and HOC-mixtures. Furthermore, under equilibrium conditions, a compound's activity is equal in all compartments, including biota. This simplifies the prediction of adverse effects, as well as its communication to decision-makers. However, currently available data on activity-response relationships of HOCs are limited to a few species, and no additional environmental stressors are considered. Therefore, the primary goal of this study is to provide a reproducible laboratory test system consisting of microcosms with artificial sediment and applicable for various sediment-inhabiting species. The sediment is loaded with HOCs to a target activity by equilibrating the organic part of the sediment (peat) with HOC-saturated water (known activity). The water is kept at saturation throughout the loading process with a reservoir of crystalline HOCs closed with a water-permeable cap. The systems have been

pre-validated with PDMS-silicone as a proxy for organic matter and three PAHs (acenaphthene, fluorene and fluoranthene). We found that the organic phase can be brought into equilibrium with the saturated water within two weeks in this system. After this time, the concentration in the silicone was within 80-120 % of the values predicted with literature-obtained silicone-water partition ratios. In parallel, water concentrations were monitored during the equilibration process. As expected, the concentration in the water phase was initially low due to the presence of the silicone HOC-sink. However, with the silicone approaching equilibrium, the water concentration came back up to saturation levels. Thus, the tested loading method is promising for the preparation of sediments for long-term ecotoxicity testing of HOC mixtures.

4.04.P-Th139 Development of a Flow-Through Water-Sediment Test System for Studies According to OECD TG 219

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The OECD TG 219 is the chronic water-sediment chironomid toxicity test with spiked water. Typically, the test is run as a static test with a single spike of a test item added to the overlying water at the beginning of the test. Five test concentrations and a control are normally used to evaluate effects on emergence, survival and growth (developmental rate). Samples of overlying water, pore water and sediment are collected at least at the beginning and end of test and optionally at intermediate time points. Following current guideline requirements, the nominal or initial measured test concentrations are to be used for endpoint derivation. However according to the "Recurring issue document" by EFSA (2019) the endpoint derivation should be based on geometric mean measured concentrations in case recovery over time is outside the 80 -120% range. In addition, a study is not considered valid in case no test item can be measured at study end. These requirements pose a problem for very instable compounds. When a test item is instable the exposure is short lived and the geometric mean for all time points results in a very low number that is not an accurate measure of exposure. This misleading low geometric mean can drive a risk assessment. A remedy to the problem of misleading low geometric concentrations is to run the test as a static renewal or flow-through test to maintain test concentrations over time. Other potential reasons for running chironomid studies under flow through conditions are variable exposure risk assessments as different exposure peaks can be tested. This allows to generate calibration and validation studies according to the EFSA TKTD scientific opinion. Testing in combination with modelling could result in the use of Tier 2C according to the EFSA AGD. Renewals are mentioned in the OECD TG 219, but the guideline does not provide methodology for how to run the test with renewals of test solutions. We have developed a flow through test system to replenish overlying water with continual additions of test solutions resulting in 5 turnovers per day. The system has been designed to minimize disturbance to the sediment and has been tested to show that the validity criteria of OECD TG 219 can be met. A test material that degrades very quickly was also tested in an abiotic system to show that test concentrations could be maintained during the test. Most aquatic labs have flow-through systems that can be easily modified to accommodate OECD TG 219 tests.

4.04 Contaminated sediment: an environmental compartment of concern (Virtual Only)

4.04.V-01 Identification of Potential Dioxin-Like Chemicals in Contaminated Sediments of the Bohai and Yellow Seas Using Effect-Directed Analysis and Non-Targeted Analysis

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In this study, identification of dioxin-like chemicals (DLCs) in polluted coastal sediments in the Yellow and Bohai seas was conducted using a combination of effect-directed analysis (EDA) and full-scan screening analysis (FSA). Firstly, aryl hydrocarbon receptor (AhR) activity of the organic extracts of sediments from a large marine ecosystem scale was screened by use of H4IIE-*luc* bioassay. Great AhR-mediated potencies were observed in raw organic extracts, aromatic fraction (F2), and subfractions of F2 (F2.6–F2.9) in sediments from Nantong, Qinhuangdao, and Yancheng. AhR responses were significantly correlated with concentrations of traditional polycyclic aromatic carbons (PAHs). However, dioxin-like PAHs could explain only < 1% of the overall induced AhR potencies. Thus, highly potent fractions (F2.6–F2.9) were analyzed using GC-QTOFMS to investigate the presence of unknown dioxin-like chemicals, which likely contribute to the overall AhR activity. A five-step prioritization strategy was applied here; ninety-two candidate compounds satisfied all of the criteria. Among these chemicals, thirteen compounds considering commercial availability of standard materials were assessed for AhR activity. Six compounds, including benz[*b*]anthracene, 6-methylchrysene, 2-methylbenz[*a*]anthracene, 1-methylbenz[*a*]anthracene, 1,12-dimethylbenzo[*c*]phenanthrene, and indeno[1,2,3-*cd*]fluoranthene were shown to be significant AhR-mediated activities. 1,12-Dimethylbenzo[*c*]phenanthrene and indeno[1,2,3-*cd*]fluoranthene were identified as novel AhR agonists, and their relative potencies values were obtained. Results of potency balance analysis showed that the six newly identified AhR agonists explained 1.3% to 100% of the total induced potencies. Thus, these compounds should be included in the monitoring of toxic substances in the future, and their sources and fate should be elucidated. Overall, combining EDA and FSA applied in the current study demonstrated the benefits of assessing the potential toxic effects of DLCs contaminated sites.

4.05 Effect modelling for regulatory environmental risk assessment: So close, and yet so far away? (Part I)

4.05.T-01 Mechanistic Population Models for Decision Support: The Importance of Good Conceptual Model Diagrams

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The use of mechanistic population models as research and decision-support tools in ecology and ecological risk assessment is increasing. This growth has been facilitated by advances in technology, allowing the simulation of more complex systems, as well as standardized approaches for model development (e.g., Population modeling Guidance, Use, Interpretation, and Development for Ecological risk assessment (PopGUIDE)), documentation (e.g., the Overview, Design concepts and Details (ODD) protocol; TRANSPARENT and Comprehensive Ecological modelling documentation (TRACE)), and evaluation (e.g., Pattern Oriented Modeling (POM)). Mechanistic population models are particularly useful for simulating complex systems, but the required model complexity can make them challenging to communicate. Conceptual diagrams that summarize key model elements can facilitate communication and understanding of complex models and increase their acceptance as decision-support tools. Currently, however, there are no consistent standards for creating or presenting conceptual diagrams, and both terminology and content vary widely. Here we argue that greater consistency in conceptual diagram development and presentation is an important component of good modeling practice, and we provide recommendations for achieving this.

4.05.T-02 Aquatic Risk Assessment of Sub-Lethal Effects With Deb-Tktd Modelling

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DEB-TKTD models are mechanistic models for the effects of constant and time-variable exposures on organisms. The EFSA provides guidance on the use of DEB-TKTD models for sub-lethal endpoints in a Tier-2 assessment, but also identified several knowledge gaps. Here we address these by (i) providing an automatised algorithm and well documented, freely available model code for a simplified DEB-TKTD model, (ii) demonstrating the fitting of all model parameters of the simplified DEB-TKTD model from data gathered from a GLP-compliant suite of experiments and an automated algorithm, (iii) providing bespoke empirical studies aimed at providing best parameter estimates while staying GLP-compliant and adhering to existing OECD guidance, (iv) state of the art case studies on three aquatic invertebrate species, and (v) example risk assessments using a moving time window approach. We show the calibration and validation of the model to bespoke experimental data on *Daphnia magna*, *Ceriodaphnia dubia* and *Hyalella azteca* under time variable exposure to azoxystrobin. The calibration experiments had long pulse exposures during the most sensitive life stages, and validation experiments followed EFSA recommendations whilst also exposing the most sensitive life-stages. The validated DEB-TKTD model was used to predict the effects of example FOCUS-SW exposure profiles on growth and reproduction. We employed a moving time-window approach and calculated the lowest multiplication factor (EP₁₀ value) from all moving time windows to identify the worst-case sections of the whole exposure profile. Our computational method ensured that sensitive life stages were exposed to whichever exposure sub-section is worst-case for them. Our method, illustrated with case studies on three aquatic invertebrate species, ensures that all life-stages are exposed to all parts of the FOCUS SW exposure profile *in silico*. Since no fixed duration can be determined as worst-case, the duration of these moving time windows was fixed to the respective OECD guideline test duration of each species. This aligns the Tier 2C1 approach with experimental Tier 2 methods. The model predictions can thus be interpreted as a range of *in silico* standardized laboratory tests, covering every possible part of the exposure profile. Efficient, open-source tools have been developed to assist the analyses; these have been largely automated but currently still require very long computation times.

4.05.T-03 Modelling Survival of Several Species Jointly in One GUTS to Reduce Animal Toxicity Testing

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Toxicodynamic-toxicokinetic (TKTD) models have been suggested as a component of environmental risk assessment (ERA) of pesticide (EFSA Aquatic guidance 2013, EFSA Wildlife draft guidance 2021). In the acute aquatic risk assessment, the reduced version of the general unified threshold model of survival (GUTS-RED) can support the assessment of species sensitivities under time-variable exposure. For this purpose, species sensitivity distributions (SSD) are constructed from GUTS-RED survival predictions for several species (at least 8 invertebrate species) exposed to realistic exposure scenarios. However, application of GUTS-RED in ERA might practically be limited by additionally required empirical efforts. At least two non-standard tests are recommended for each species to validate a species-specific GUTS-RED (EFSA TKTD Opinion 2018), which amounts to an extra of 16 invertebrate tests for a GUTS-RED-based SSD. Here, we present a novel approach to jointly model species in the GUTS-RED framework. The joint model is based on recently established relations among TD-parameters that are shared across species, if these employ common receptors and pathways in their response to a toxic compound. Calibration of the joint GUTS-RED is eased compared to the taxa-specific models, as it exploits additional information on the TD-parameter relations shared in the taxonomic group. In particular, for 8 invertebrate species, the number of TD parameters that need to be calibrated reduces from 2 taxa-specific parameters for each of 8 species to 1 taxa-specific parameter per species + 1 joining parameter capturing information across the taxonomic group. We show that GUTS-RED-IT jointly calibrated to the taxonomic group match or outcompete species-specific GUTS-RED-IT in terms of parameter calibration and survival predictions. We suggest that the reduced degrees of freedom in the joint model provide the potential for more reliable model predictions. Further, as the joint GUTS-RED models taxa at the level of their taxonomic group, it can be validated from a representative subsample of the taxa, which reduces animal toxicity testing.

4.05.T-04 Predicting the Effect of Food Level on Individual and Mixture Toxicity of Prochloraz and Benzalkonium Chloride in *Caenorhabditis elegans*: Comparing Dynamic Energy Budget and Classic Concentration-Response Modelling

Approaches

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Simplified DEB models coupled with toxicokinetic and toxicodynamic (TKTD) modules are a powerful tool for understanding toxic effects of multiple endpoints over time. However they have not yet been implemented widely in risk assessment due to model complexity and data requirements. A recently updated DEBtox model was implemented to analyze sublethal toxicity data from experiments designed for dynamic modelling for two chemicals, prochloraz and benzalkonium chloride (BAC), and one environmental stressor (food stress) in the nematode model organism *Caenorhabditis elegans*. This study was conducted with three aims; to test performance of the experimental design, to compare interpretation of effects between the DEBtox and concentration-response analysis, and finally to analyze the predictive power of DEBtox extrapolations for single chemical and mixture toxicity under food stress. The 96 hour experimental design for growth and reproduction endpoints in *C. elegans* performed well in providing data for dynamic modelling. Growth and reproduction over time were successfully modelled using the DEBtox model. According to the best-fitting DEBtox model, the physiological modes of action (pMoA) were growth for prochloraz and maintenance for BAC, which is in accordance with known modes of action of prochloraz and BAC documented in the literature. Functional response decreased with decreasing food level, in line with DEB theory assumptions. The DEBtox model could predict effects of food level alone and in combination with prochloraz quite accurately, which was not possible with concentration-response analysis. Independent action could predict the mixture toxicity of prochloraz and BAC fairly well (IA prediction: 44% effect; measured mixture toxicity: $33.56 \pm 6.5\%$ effect), but not at low food level, when mixture effect increased to 75% (± 3.88). The DEBtox model predicted the effect of prochloraz at low food quite well (57.78% effect predicted compared to $46.76 \pm 11.33\%$ measured). However the DEBtox model underestimated the effect of BAC and the mixture prediction at low food was the same as for prochloraz alone. DEBtox models can be powerful tools to understand chemical stressors in combination with environmental ones, but they require further testing to make reliable predictions for multiple stressor interactions.

4.05.T-05 The Integrative Honeybee Colony Model BEEHAVE: Lessons Learned

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Honeybees play an important role in the environmental risk assessment of pesticides. While the effects of pesticides on honeybees have been intensively researched in terms of development, behaviour, and mortality of individual bees under controlled conditions, it is still largely unknown how these effects play out at the colony level in real landscapes. Since controlled field experiments at the landscape scale over one or more entire seasons are not possible, mechanistic modeling appears to be the only way to better understand and quantify colony-level impacts. The BEEHAVE model, published in 2014, includes a spatiotemporal representation of nectar and pollen availability in the landscape, foraging, inhive dynamics including brood care, consumption of nectar and pollen, and virus transmission by *Varroa* mites. Although the model does not include a pesticide module, it is the first to allow the study of multiple stressors and is based on first principles that can translate environmental conditions, including weather, into colony performance and survival. Beehave was published open-access and deliberately implemented in a programming language that is free, transparent, and easy to learn, so that all sectors involved, including academia, beekeepers, industry, and regulators, can use it as a tool that allows to integrate different questions, points of view, and results. Since 2014, 18 applications of BEEHAVE have been published. We examined these applications in terms of the issues addressed, potential additions to the model, and key lessons learned. BEEHAVE has been used to examine the effects of forage gaps and forage quality, lethal and sublethal effects of pesticides, development of thresholds for risk assessment, and the evaluation of multiple stressor interactions. While most early applications used stylized landscapes and hypothetical pesticide effects, more recent studies have focused on validating field studies. Specific pesticide modules have been developed, as well as a module for *Varroa* control. In a study conducted by EFSA, BEEHAVE was parameterized for different sites in Europe to generate reference dynamics for bee colonies that were not affected by pesticides. Although the complexity of factors affecting honey bee colonies makes it impossible to summarize the lessons learned in a concise manner, BEEHAVE has proven to be a valuable tool for better understanding and predicting honeybee colony dynamics and the impact of various stressors.

4.05 Effect modelling for regulatory environmental risk assessment: So close, and yet so far away? (Part II)

4.05.T-06 Assessing the Population Relevance of Adverse Effects of Endocrine Disrupting Chemicals on Individuals: A Practical Modelling Approach

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The presence of endocrine disruptors (EDs) in the environment continues to be a cause for concern for wildlife given some have been shown to cause adverse effects on organisms at low exposure concentrations. However, there is still a significant lack of understanding on the potential effects of EDs on populations and communities. This fundamental shortfall has real-world environmental management and regulatory implications, where the aim is to protect populations. The ECHA/EFSA 2018 guidance on performing an assessment for ED of chemicals states that any adverse effect on an individual recorded in a laboratory experiment (e.g. reduced fecundity) is assumed to be an adverse effect on a population unless additional evidence is provided, albeit that population level effects are established for very few EDs. Population modelling is proposed as one of these additional lines of supporting evidence, however, statements in the EFSA/ECHA ED guidance on the practicality of how to use population

models in this context are absent. Crane et al. (2019) suggested a hazard-based framework using population modelling within an adverse outcome pathway (AOP). We present a methodology to assess the population relevance of ED exposure using population modelling in line with this AOP framework. This includes critical decisions regarding model choice, data (effects and exposure) and how to integrate the two (hazard- or risk-based) approaches. We provide a case study with prochloraz, an imidazole fungicide that has been shown to have feminising effects in fish (acting as both an antioestrogen and an anti-androgen) to illustrate how this approach could be applied and evaluate how different decisions may alter the population response. Two fish population models for trout (inSTREAM) and stickleback that met requirements for development and validation were identified and used in the study. Effects data extracted from the literature was combined with exposure profiles generated with FOCUS SW. Population effects were observed in some scenarios (hazard-threshold) and not others (dose-response) demonstrating different outcomes that would then invoke making different decisions. The population responses of both species exposed to prochloraz was similar, indicating that for this compound the use of conservative effects / exposure decisions may over-ride differences in life-history and vulnerability to ED exposure. This study indicates that models can be used to effectively support the evaluation of the effects of EDs on fish populations and that the proposed method may be adapted for regulatory purposes. However, further work and consensus are required to ultimately establish an agreed approach for this.

4.05.T-07 Predicting Combined Effects of Chemical Stressors: Population Effects of Mixtures With Deb-Ibm

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The current risk assessment framework contains discrepancies between protection goals and the information received from risk assessment. Uncertainty gaps exist when using lab test results as proxy for the complex environment. Mechanistic population models can bridge the gap between individuals and populations. Additionally, mixture toxicity can be included in mechanistic models to predict combined effects of chemical stressors. Our main research questions here are: in a multiple chemical stressor environment, which patterns of effects are observed on populations exposed to mixtures, and can we predict those patterns with DEB-IBM? We used an individual-based model implementation of the dynamic energy budget theory (DEB-IBM) for *Daphnia magna* to design a population experiment where we tested combinations of physiological modes of action (PMoAs). Our hypothesis is that combined effects emerge from the linking of DEB energy flows. Mixture combinations and concentrations were selected based on the DEB-IBM. We tested the effects of four organic compounds to *D. magna* populations: pyrene, dicofol, ?-HCH, and endosulfan. A 17-week experiment was conducted consisting of two phases: a constant-exposed period (first 11 weeks, exposure initiated after 1 week, lasting for 7 weeks, followed by 3 weeks recovery), and a pulse exposure period (last 6 weeks, including a 3-day acute pulse, and final recovery). We validated the DEB-IBM with the population data. With DEB-IBM, mixture effects at the population were predicted, in line with the observation of the population experiment. Moreover, these are blind predictions at the population level, with a calibration on single-substance, individual-level data only. The DEB-IBM accurately predicted the effects during the constant phase, the recovery phase, and the acute phase with great accuracy. After the acute pulse, the model became unreliable due to stochasticity in a population-level context. Synergisms were observed in the population data, which were accurately predicted by the DEB-IBM as well. This confirmed our initial hypothesis where mixture toxicity emerges from the linking of DEB energy flows. Moreover, the DEB-IBM performed better in predicting mixture toxicity than statistical models (such as the independent action [IA] model). To use IA, population data with single substances is required, whereas DEB-IBM predicts mixture population effects based on prior model calibration with single-substance individual-level data.

4.05.T-08 Inferring Chronic Pesticide Effects From Long-Term Daphnia Population Experiments With a Stochastic Population Model

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Identifying sublethal pesticide effects on populations is challenging. Long-term population experiments can help to assess chronic toxicity. However, population experiments are laborious and subject to stochasticity (demographic, environmental, and genetic). Therefore, large variation among a limited number of replicates can lead to non-conclusive results based on classical statistical analysis. Model-based analysis can support the interpretation of such experimental data to quantify sublethal pesticide effects. The goal of such an analysis is to identify the most important processes that determine population dynamics and lead to stochasticity and describe them in the model. The main challenge for this task is choosing an adequate level of model complexity. Models should be as simple as possible and as complex as necessary for an adequate description of the system. In this study, we tackled this challenge by using a systematic model selection process based on a nested design of the model structure and Bayesian inference to identify mechanisms from population experiments. We used a stochastic, age structured population model applied to data from long-term population experiments with *Daphnia galeata* in 1L aquaria with and without chronic pesticide treatments (Diazinon and Diuron) at sublethal concentrations. We tested different formulations of fecundity and mortality and compared the inferred parameter estimates from the different treatments. Posterior analysis helped to choose an adequate model description for life-history characteristics under the specific experimental conditions: a zero-inflated negative binomial distribution for fecundity and mortality without density dependence. For the Diazinon treatments, a comparison of the inferred posterior parameter distributions indicated the need for a time-dependent mortality rate that increases with time, indicating cumulative chronic toxic effects of Diazinon on the *Daphnia* populations. With this study, we demonstrate how we can use stochastic models to infer mechanisms from population data to help identifying sublethal pesticide effects. The model-based analysis can also support the optimization of the experimental design of future studies.

4.05.T-09 Validating Models With Experiments, or Interpreting Experimental Data With Models - the Advantages of Using Ibm-Based Approaches to Modelling Aquatic Mesocosms

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In recent years, individual-based population models (IBMs) have been increasingly used in ecological risk assessment, often coupled with TKTD effect models. Integrated within ecosystem models, they can be powerful tools for predicting species population dynamics for different environmental scenarios. This study is part of a larger ring study to compare four aquatic system models of different model structure and evaluate whether such model approaches can be used to simulate aquatic mesocosms, and thus might be acceptable in the regulatory context of pesticide risk assessment in the future. This ring study also provides an opportunity to further develop data sampling in aquatic mesocosms for the purpose of model development and testing. Here we used the ecosystem model framework StoLaM+, which is a coupling of the lake model StoLaM including IBMs for pelagic crustaceans, an IBM for *Chaoborus crystallinus*, and the aquatic stream community model STREAMcom also including a variety of IBMs for benthic macroinvertebrates. Data from seven outdoor aquatic mesocosm studies are available for the project. We exemplarily analysed mesocosm data with different levels of complexity (including either just one or multiple developmental stages) and methodology (with or without area reference to the entire mesocosm) for selected aquatic species from pelagic and benthic communities (copepods, *Chaoborus*, *Cloeon*, *Asellus*) using IBMs. Due to their individual-based organisation, IBMs are designed to easily provide multiple quantitative endpoints for a modelled population, e.g., the number of individuals at different life stages. Especially in cases where different experimental methods have been used for different life stages of the same species, this model approach can reveal gaps in the consistency of population field data. Additionally, conversion factors for abundance measurements can be estimated for extrapolation to the entire system using IBMs, as long as at least one endpoint has a quantitative relationship (e.g., per area or volume) to the investigated ecosystem. This study shows that a comparison of model results should not automatically lead to a unidirectional fit of the models to available complex data sets in every case, but should include the possibility of performing an alternating plausibility check between measurements and simulations, depending on the specific type of measured data and methods used.

4.05.T-10 Comparative Ecosystem Modeling Study for Assessing Pesticide Risks in Aquatic Mesocosms

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Outdoor aquatic mesocosms are artificial ecosystems used for higher-tier pesticide risk assessments to assess the effects of pesticides across a wide range of organisms and to capture indirect effects mediated by the food web. Mesocosms provide more realistic estimates of risks compared to single-species laboratory-based standard toxicity studies. Aquatic system models (ASMs) have been developed to simulate mesocosm systems and can extend pesticide risk assessment through applying scenarios corresponding to conditions untested in the empirical systems, including time-variable exposures. We are presenting a comparative modeling (“ring”) study with four previously published ASMs (Streambugs, AQUATOX, CASM, and StoLaM+StreamCom). With the ring study, we aim to assess whether the artificial mesocosm systems can be simulated with ASMs. In addition, the study is an opportunity to gain insight into and inform data collection from the mesocosms themselves and allow for comparison between available ASMs. The goal of the study is to calibrate and validate the models with the same set of available mesocosm data. Mesocosm data are available from untreated control mesocosms from seven studies conducted in four different years at the FNU Research Centre Neu-Ulrichstein, Germany. We reviewed these data for patterns and consistencies across studies, and derived calibration and validation criteria to compare with the model outputs. Four of the available control data sets were used for calibration by all models. The remaining three data sets were used for validation in a separate step. The ring study provides a unique opportunity to compare previously published ASMs and assess whether they can be used to simulate the transient ecosystem responses in mesocosms. The ring study attempts to develop and establish ASMs as virtual mesocosms acceptable in the regulatory context of pesticide risk assessment.

4.05 Effect modelling for regulatory environmental risk assessment: So close, and yet so far away? (Virtual Only)

4.05.V-02 Evaluating the Influence of Growth Rate in Macrophyte Risk Assessment

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The use of plant protection products can result in a variety of exposure profiles in edge-of-field water bodies. TKTD models allow to realistically assess the effect of this time-variable exposure on macrophytes. EFSA PPR Panel (2018) considers the *Lemna* TKTD model of Schmitt et al. ready to be used in regulatory risk assessment, paving the way for a broad use of this model in risk assessment. However, it has been argued that under time-variable exposure regimes, the *Lemna* model may not be representative for macrophyte species such as *Myriophyllum*, due to their slower growth: e.g., while a minimum doubling time of frond numbers of 2.5 days is required in a *Lemna* test, the minimum doubling time of total shoot length in a *Myriophyllum* test is 14 days. It has

been reasoned that fast *Lemna* growth would allow faster recovery during periods with low or no exposure. On the other hand, earlier research using global sensitivity analysis on the Lemna model (Klein et al., SETAC Europe 2021) has shown that for typical *Lemna* growth rates, growth rate inhibition is rather insensitive to changes in growth rate. To get a better understanding of the influence of the growth rate on macrophyte risk assessment, we perform simulations with the *Lemna* TKTD model under laboratory growth conditions and time variable exposure. We investigate the effect of different baseline growth rates on growth rate inhibition for different sets of parameters and exposure regimes. We discuss the mechanisms involved, the extent of effects, and the implications for macrophyte risk assessment.

4.05.V-03 Simple Debkiss-Based TKTD Model for Chironomids

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Toxicokinetic-toxicodynamic (TKTD) modelling is receiving increasing interest in the regulatory risk assessment of pesticides. TKTD models explicitly consider the factor 'time', and are based on mechanistic principles, thereby allowing extrapolation across (time-varying) exposure scenarios. For sub-lethal effects, generic and practical TKTD models should be based on energy-budget considerations. Dynamic Energy Budget (DEB) theory is well established in this area, and practical, simplified frameworks such as DEBKiss are derived from it. Despite the obvious advantages of DEB-based TKTD modelling, there are species groups whose life histories do not fit well with the generic standard models. The insects are a particularly prominent group for which this is the case. These animals split up their life cycle into a growing, non-reproducing, phase (the larva) and a non-growing, reproducing, phase (the adult). For holometabolous insects, this split is accompanied by a dramatic morphological metamorphosis. In many species the adult does not even feed, or at least: does not have to feed to produce eggs. Several attempts at DEB modelling of insects have been made. However, these models are too complex to apply to risk assessment (at least, given the typical data sets that are available) and tend to yield unrealistic responses under stress (e.g., food limitation). In this study, we set out to develop a novel, simple, DEBKiss-based TKTD model for chironomids (nonbiting midges). The model should be simple enough to be parameterised from the limited data from standard experimental tests, extended with several length measurements in the larval phase. This implies sacrificing some mechanistic principles in favour of more pragmatic descriptive elements. However, the crucial test of the model will be to see whether it is able to extrapolate between different time-varying exposure scenarios. In this presentation, we will explain the model structure and report on the model testing against data from dedicated experimental tests.

4.05.V-04 The Importance of Confidence Limits for Ecotoxicological Endpoints

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In Europe, Tier I ecotoxicity tests using standard species are a regulatory risk assessment requirement for all chemicals prior to commercial use. The most common statistical endpoint used in acute and chronic ecotoxicological studies are Effect Concentration (EC_x) values. These describe the concentration at which the x percent of the organisms are affected for any given parameter, e.g. mortality, i.e. an EC₅₀ would be where 50% of all tested organisms had died. In recent years, there has been a requirement for the provision of both EC₁₀ and EC₂₀ values in addition to the traditional EC₅₀ values. Therefore, older study data is routinely re-analysed to generate these values to be submitted to the regulatory authorities for current environmental risk assessments. These re-analyses should also include an assessment of the reliability of each endpoint. This is usually carried out by investigating the corresponding confidence limits. Although confidence limits are a key indicator of the reliability of the data, they can sometimes be overlooked, or their importance misunderstood. Here, we provide an overview on the current guidance for calculating EC_x values, and their confidence limits, to provide an insight on how confidence limits can be used to identify a reliable EC_x endpoint. We will also highlight the standard species accepted endpoints detailed in the OECD test guidelines.

4.05 Effect modelling for regulatory environmental risk assessment: So close, and yet so far away? (Poster)

4.05.P-We176 Heart Rate As an Early Warning Parameter for Mortality in Fish Embryos Exposed to Ionisable Substances

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Environmental risk assessment of organic chemicals usually does not consider pH as a key factor. Hence, most substances are tested at a single pH only, which may largely underestimate the toxicity of ionisable substances with a pK_a in the range of 4–10, with differences in toxicity between pH's of up to a factor of 2000. Thus, the ability to consider pH-dependent toxicity is essential for a more realistic assessment. We used *Danio rerio* embryos exposed to ten ionisable substances (the acids diclofenac, ibuprofen, naproxen and triclosan and the bases citalopram, fluoxetine, metoprolol, propranolol, tramadol and tetracaine) at four external pH levels, investigating the endpoints mortality (LC50) and heart rate (EC20). Dose-response curves were fitted with an ensemble-model to determine the true uncertainty and variation around the mean endpoints, and with relative model quality estimation based on the Akaike Information Criterion (AIC). This resulted in more robust EC_x estimates with lower 'standard errors' as compared to randomly selected individual models. We detected a high correlation between mortality (LC50) at 96 hpf and reduced heart rate (EC20) at 48 hpf for all compounds and all external pH levels (r=0.98). Moreover, the observed pH-dependent effects were strongly associated with log D and thus, likely driven by differences in uptake (toxicokinetic) rather than internal (toxicodynamic) processes. Prospectively, the *a priori* consideration of pH-dependent effects of ionisable substances might make testing at different pH levels redundant, by applying a respective safety factor, while the endpoint of mortality might

even be replaced by a reliable sublethal proxy that could reduce the exposure of the test species, while accelerating the evaluation process.

4.05.P-We177 Solitary Bees in Agricultural Landscapes: SolBeePop, a Population Modeling Approach

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With their large diversity of species, solitary bees are important pollinators of crops and native plant communities. In agricultural landscapes, they can potentially be exposed to pesticides, and thus, are considered explicitly in risk assessments of pesticides. The managed Western honey bee (*Apis mellifera*) is currently used as surrogate across bee species in these assessments. Fundamental ecological differences result from the social and solitary life styles, introducing uncertainty in the estimation of pesticide risk to solitary bee populations. We developed the model SolBeePop, a population model for solitary bees in agricultural landscapes. The model can simulate a variety of species by using species-specific traits as model parameterizations. Model parameterizations for the modeled species (*Osmia bicornis*, *O. cornifrons*, *O. cornuta*, *O. lignaria*, *Megachile rotundata*, *Nomia melanderi*, and *Eucera (Peponapis) pruinosa*) were compiled from the literature whereby data availability varied by species. The model can simulate the diverse life cycles of the species and can be used to explore the importance of uncertainties in data to the population dynamics. We calibrated and validated the model with control data from semi-field studies conducted with *O. bicornis* and *O. cornuta*. The model can successfully capture population dynamics observed in these studies. Further developments include the implementation of an exposure and effect module which will make it possible to simulate exposure and population-level effects in the context of realistic landscape scenarios. Thus, this model will become a valuable tool for higher-tier pesticide risk assessments of solitary bees in agricultural landscapes.

4.05.P-We178 An Environmental Risk Assessment Case Study on *Ceriodaphnia dubia* Analysed by 'DEB-TKTD'

Modelling

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Toxicokinetic-toxicodynamic (TKTD) modelling is receiving increasing interest in the regulatory environmental risk assessment of chemicals. This type of mechanistic model integrates all available data in a single framework. In particular, it allows performing refined risk assessment by extrapolating from laboratory results to relevant Tier 2C refined exposure scenarios, based for instance on FOCUS-sw predictions. Dynamic Energy Budget models coupled with TKTD modules (DEB-TKTD) are the leading approach to assess sublethal effects of chemicals on individual organisms. EFSA recognised, in its 2018 opinion, several strengths of DEB-TKTD modelling, but also highlighted some challenges. Particularly, EFSA stated that the “lack of published examples of DEBtox [DEB-TKTD] models for pesticides and aquatic organisms [...] results in the conclusion that these models are not yet ready for use in aquatic risk assessment for pesticides”. The aim of this study is to address this gap by providing a state-of-the-art example on a standard test species (*Ceriodaphnia dubia*) in aquatic risk assessment. This was achieved by following the framework proposed in the Scientific Opinion. In the first step, bespoke empirical studies based on OECD 211 guidelines were designed to allow obtaining the best estimate and identification of all relevant model parameters while staying GLP-compliant. A DEB-TKTD model, DEBtox2019, was then calibrated on these data and used to inform the design of the validation experiment. Once validated, the model was used to perform predictions for a refined exposure scenario based on FOCUS-sw exposure profiles. A moving time-window approach was used to perform the environmental risk assessment. This case study highlights and discusses how DEB-TKTD can be used in the aquatic environmental risk assessment of pesticides.

4.05.P-We179 Deb-Tktd Modelling for the Environmental Risk Assessment of Pesticides on Chironomids

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The current regulatory environmental risk assessment of plant protection products (PPP) is often based on controlled laboratory experiments with constant or refined simple pulse exposure scenarios. While this approach has the benefits of simplicity, the tested exposure patterns are far from the real-world ones. In addition, the summary statistics derived from these traditional approaches do not allow, for instance, meaningful extrapolations to non-tested exposure patterns or to different exposure durations. Mechanistic modelling and, in particular, toxicokinetic - toxicodynamics (TKTD) models have the potential to overcome these challenges. These types of models make holistic use of all data available by integrating them into a unique framework. Dynamic Energy Budget models coupled with TKTD modules (DEB-TKTD) are the leading approach to assess sublethal effects of PPP on individuals. However, to date, no satisfactory DEB-TKTD-based model for use in regulatory risk assessment was available for chironomids. This challenge has now been overcome. We here present a case study with *Chironomus dilutus* exposed to a pesticide. Tailored OECD compliant life-cycle experiments were performed for both model calibration and model validation. A model based on a simplified DEB model was used to analyse these data according to the Scientific Opinion on TKTD modelling for EFSA. Ultimately, the validated model can be used to perform Tier 2C refined exposure environmental risk assessment.

4.05.P-We180 Can 'DEB-TKTD' Modelling Enhance Environmental Risk Assessment? - a Case Study With *Daphnia magna*

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Yes, it can! Traditional environmental risk assessment of plant protection products (PPP) is usually based on controlled laboratory experiments. These experiments are often conducted under constant or refined simple pulse exposure scenarios. These experimental conditions are often far removed from real conditions where complex time-variable exposure scenarios are the norm. Such challenges can be overcome thanks to mechanistic modelling. Mechanistic modelling is recently gaining interest in regulatory environmental risk assessment as it allows considering, within an integrated framework, more realistic environmental scenarios. In particular, toxicokinetic - toxicodynamic (TKTD) models make a holistic use of all data possible, by integrating them in a single framework. Dynamic Energy Budget models coupled with TKTD modules (DEB-TKTD) are the leading approach to assess sublethal effects of PPP on individuals. The EFSA Scientific Opinion on TKTD modelling recognises DEB-TKTD modelling as a potentially powerful tool for use in environmental risk assessment. It also identified that DEB-TKTD models are not yet ready for use in risk assessment as “sufficiently calibrated and validated DEBtox models for pesticide and aquatic organisms were not yet available in the open literature”. We here address this limitation by presenting a case study for *Daphnia magna* exposed to a pesticide. This case study followed the framework proposed by EFSA. A DEB-TKTD model was calibrated on bespoke studies based on the OECD 211 *D. magna* reproduction test. In a second step, the model was used to inform the design of the validation experiment with repeated pulsed exposure. The duration between pulses was varied across treatments to align with EFSA recommendations to validate toxicologically dependent and independent exposures. The model was then validated against the newly produced validation dataset. In a final step, the validated DEB-TKTD was used to perform an environmental risk assessment of relevant exposure profiles using a time-window approach. This case study showcases the advantages and challenges of using DEB-TKTD for risk assessments.

4.05.P-We181 On the Use of 'DEB-TKTD' Modelling for Regulatory Environmental Risk Assessment of Pesticides - a Case Study With H. Azteca

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Mechanistic modelling and, in particular, toxicokinetic-toxicodynamic (TKTD) modelling, has received increasing interest in the regulatory environmental assessment for more than 10 years now. In its 2013 Aquatic Guidance document, EFSA already stated that TKTD models “may be used to predict the time course of effects of time-variable exposures”. This increased interest has culminated in the publication in 2018 of the “EFSA Scientific Opinion on the state of the art of Toxicokinetic / Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms”. Traditional environmental risk assessment of chemicals is usually based on controlled laboratory experiments. These experiments are often conducted under constant or limited pulse exposure concentrations. These experimental conditions are often far removed from real conditions where complex time-variable exposure scenarios are the norm. Mechanistic modelling, and in particular TKTD modelling, allows considering within an integrated framework more realistic environmental scenarios. Despite its numerous strengths and advantages, EFSA considers that the use of TKTD modelling to analyse sub-lethal effects, i.e., dynamic energy budget models coupled with the TKTD module, is not yet ready for regulatory risk assessment. This conclusion mainly arises from the lack of published examples and case studies. In this study, we aimed at overcoming this challenge by presenting a case study for the standard test species *Hyalella azteca* exposed to a pesticide. We used a well-recognised DEB-based model, DEBtox2019, and designed bespoke empirical studies aimed at providing the best parameter estimates while staying GLP-compliant and adhering to existing guidance. Our study follows the recommendation of the EFSA scientific opinion on TKTD modelling and shows and discusses the advantages and challenges of using DEB-TKTD for risk assessments.

4.05.P-We182 DEBgen: A Generalised Model Formulation to Incorporate Common Deviations From the Standard Model of Dynamic Energy Budget Theory

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The Dynamic Energy Budget (DEB) theory has become a popular starting point when modelling the life history of individual organisms, e.g., as physiological units of an individual-based population model (IBM). Compared to other, often purely data-driven, *empirical* models, DEB models use a coherent framework of mechanistic rules – on the level of bioenergetic budgets – that respect the law of conservation of energy. As a result, these models are achieving increasingly high levels of trust, which is a key requirement for their potential use in environmental risk assessment. Another advantage of DEB models is their generic nature in the sense that theoretically any species can be modelled using the same set of bioenergetic rules and corresponding model equations, with only the deviations in parameter values accounting for inter-species differences. And while this appears to be true for many species, numerous exceptions, i.e., species that deviate from the standard DEB model, have been identified in recent years. This has led to the development of *typified models*, typically adding additional life stages (e.g., with an accelerated growth), in which they deviate from the standard model. While these typified models are relatively well documented, ready-to-use model implementations are scarce when compared to the standard model. Starting from a standard model and adapting it to a typified model is a rather error-prone task. Starting from a different typified model can be even more confusing since life-history events of the same name (specifically *metamorphosis*) can have different meanings (i.e., they trigger different rule changes) among the typified models. Here we argue, that the most commonly used typified models do not actually contradict the standard DEB model but can rather be seen as extensions. Conversely, one can think of a more general model formulation of which the standard DEB model and many typified models merely represent special cases. We present the model framework DEBgen, which so far covers the model types *std* (standard model), *abp*, *sbp*, *abj*, and *asj*. Further extensions (e.g., incorporating *hep* and *hex*

models) are also planned. A particularly useful application of DEBgen is its use as a building block in community models that incorporate multiple species described with different typified models. Thus, one would only need to implement a single DEB model structure instead of creating new code for each DEB model type.

4.05.P-We183 To Combine or Not to Combine Data Sets for GUTS Calibration

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Toxicokinetic-toxicodynamic (TK-TD) models allow for the integration of exposure and effects in an environmental risk assessment context. The General Unified Threshold Model of Survival (GUTS) is a particularly promising tool for predicting lethal effects under time variable exposure conditions. Before being used in risk assessment, GUTS models need to be parameterized and validated based on different toxicity data sets. The EFSA Scientific Option on TK-TD modelling (<https://doi.org/10.2903/j.efsa.2018.5377>) suggests that GUTS is calibrated on experimental data under constant and/or time variable exposure and preferably validated on data from different time variable exposure scenarios. The calibration data, but not the validation, will influence the parameter estimates and their uncertainty and thus the risk assessment endpoint calculated by GUTS (such as the Lethal Profile LPx). In particular, the parameter uncertainty will directly depend on the level of ‘information’ in the calibration data – generally speaking, the better the information, the better the parameter estimates. In most cases, calibrating GUTS based on a dataset from a single study will be sufficient for predicting risk assessment endpoint with an adequate confidence level. We will however illustrate, using data sets from toxicity tests with *Chironomus riparius* and the insecticide flupyradifurone as an example, that parameter uncertainty may decrease (rather than increase) when combining different types of toxicity studies for GUTS calibration.

4.05.P-We184 Can You Reduce Validation Effort When Jointly Calibrating GUTS Models for Multiple Species?

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In regulatory risk assessment of Plant Protection Products (PPP), an option for endpoint refinement using effect modelling has been included in guidance documents since 2013 (Tier IIC; EFSA Aquatic Guidance 2013). A detailed description on the type of models that are fit for the regulatory process, their application, and underlying data requirements were outlined and discussed in detail in the EFSA Opinion on TKTD modelling (2018). For lethal effects there is a clear recommendation for the parameterization of the (reduced) GUTS model. Although PPP notifiers are often very interested in the modelling approaches, in our experience, many are hesitant to ultimately choose an effect modelling approach to address their regulatory questions. This is mainly due to the uncertainty surrounding regulatory acceptance and the high costs associated with calibration and validation experiments, particularly if a large number of species are involved. Often, existing data sets from previous submissions do not include the necessary information to be re-used for model parameterization. The lack of information in some studies may be circumvented by calibrating GUTS jointly using multiple species data sets if parameters correlate. Here, we propose conducting a reduced number of non-standard higher tier experiments, for a set of key species to validate the multi-species GUTS parametrization. We highlight where the uncertainties of this approach within a risk assessment lie, as well as the potential advantages and knowledge gains.

4.05.P-We185 Refining Aquatic Risk Assessment With GUTS Modelling: The Right Tool for the Job?

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In recent years toxicokinetic-toxicodynamic (TKTD) approaches have been developed to describe the effects of chemical substances on organisms over time. TKTD models represent real-world scenarios, such as time-variable pulsed exposure profiles, and hence overcome the limitation of conventional toxicity indicators which are commonly estimated at the end of an exposure period that assume constant exposure concentrations over time. Among the TKTD models, the General Unified Threshold model of Survival (GUTS) is considered ready-to-use in ERA by the European Food Safety Authority (EFSA 2018). In the tiered approach for pesticide risk assessment, GUTS is recommended as powerful Tier-2C refinement tools to assess the survival probabilities of aquatic organisms under realistic time-variable exposure profiles (i.e. by integrating the results from fate models). However, despite being structurally simple (TKTD models consist of three or four equations that link external concentrations to survival effects over time) and supported by easily accessible and user-friendly modelling tools (e.g. OpenGUTS and MOSAIC), the approach has seen limited use in regulatory environmental risk assessments. To determine the cause of this mismatch between their theoretical advantages and practical uses, we present an overview of the commercial viability of GUTS modelling in regulatory applications. In particular, GUTS approach is compared to the other options available to solve an aquatic risk assessment (e.g. multiple species testing approaches at Tier 2A and Tier 3 and Tier 2B pulsed exposure studies) in order to highlight the benefits of using a TKTD modelling approach as well its practical limitations in a pesticide dossier submission. For commercial viability, different risk assessment approaches can be combined if the potential use of TKTD is considered early on (as in Tier 1 testing and Tier 2B pulsed exposure studies). Here we present a case study where a TKTD approach was considered but not pursued due to cost implications. We thus define the characteristics of standard test studies that would make GUTS a routine part of regulatory submissions, whilst also identifying other refinement options that are appropriate in alternative situations.

4.05.P-We186 The Application and Limitations of Exposure Multiplication Factors in Sublethal Effect Modelling

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Toxicokinetic-toxicodynamic (TKTD) models can be powerful tools in many areas of ecology, including environmental risk assessment (ERA) of substances such as plant protection products. To facilitate this use, exposure multiplication factors (EMFs, sometimes known as multiplicative margin of safety values) have been recommended by EFSA [1]. An EMF scales the whole exposure profile being assessed. The aim of the risk assessment is to find an EMF which - when applied to an exposure profile - results in some specified percentage effect relative to the control at the end of the exposure profile. This is known as the LP_x or EP_x (for sublethal effects). The core assumption of the approach is that increasing the scaling of a given exposure profile will always produce more extreme effects. Unlike experimental approaches, TKTD models offer an opportunity to interrogate this assumption in a mathematically rigorous manner. For the GUTS framework, and a chosen DEB-TKTD model we show the results of seeking a proof that LP_x or EP_x values exist and are unique for any x% effect and any non-zero exposure profile. We find necessary conditions for existence of these values and show that, in the majority of cases, the LP_x (EP_x) for an exposure profile is unique if it does exist. However, in DEB-TKTD models, there are certain model configurations where higher EMF values can have reduced effect, which also means that there may be more than one EP_x value for the profile. In other words, scaling up an exposure can reduce the effects observed at the end of the exposure profile. Assessing these modelling techniques with such rigour provides the utmost confidence in their use and their limitations. Most importantly, this work reveals both scientific and regulatory questions of great importance and interest. When non-uniqueness of the EP_x is a concern in ERA, algorithms may report a higher (but equally correct) value as the EP_x. Slower methods may need to be used to estimate the lowest EP_x. This unexpected key finding could naturally lead into many new investigations, for example attempts to replicate the modelling result *in vivo*.

4.05.P-We187 Exploring GUTS in the Environmental Risk Assessment of Pesticides: A Case Study

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Pesticide regulations are based on environmental risk assessment that incorporates information on the fate and transport (exposure) and ecotoxicological effects of a chemical on exposed organisms. At Tier 1, effects data are based on standardized laboratory studies (i.e. single active substance, constant concentration and few model organisms) which do not take into account the time-variable exposure profile of the chemicals. Toxicokinetic (TK) and toxicodynamic (TD) models have been developed to improve the environmental risk assessment of chemicals by describing the effects of chemical substances on organisms over time. Recently, the General Unified Threshold model for Survival (GUTS) has been judged to be “ready to be used in risk assessment” by the Panel on Plant Protection Products and their Residues (PPR) of the European Food Safety Authority (EFSA). However, despite the advantage of TKTD modeling, this approach is still underutilized within the context of environmental risk assessment. This presentation looks to answer the question of “why?” by presenting GUTS modelling for two active substances that relied on mesocosm data (Tier 3) for approval for use in the EU by EFSA. Our aim is to discuss the potential reasons for the applicability and common use of GUTS models in the EFSA tiered scheme for the aquatic invertebrates acute risk assessment, that may inform the selection of appropriate refinement methods in the future.

4.05.P-We188 Mixture Effects of Two Insecticides on the *Enchytraeus crypticus* Life Cycle Predicted With a Deb-Tktd Model

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The Dynamic Energy Budget theory (DEB) enables modelling the effects of chemical stressors on the organism life cycle through the coupling with toxicokinetic-toxicodynamic (TK-TD) models. The DEB-TKTD framework also has the potential to predict effects in complex situations such as mixture effects. Hence, DEB-TKTD models are of great interest for pesticide risk assessment, but so far insufficient applied case studies are available in the scientific literature to assess their reliability. For soil organisms, these models are even scarcer, and currently, none can be found in the literature for enchytraeids, despite the fact that they are model organisms in soil ecotoxicology. We here present a DEB-TKTD model for the model species *Enchytraeus crypticus* that enables us to predict mixture effects of two insecticides. Hatchlings of *E. crypticus* were exposed in the standardized LUFA 2.2 soil, to cypermethrin and imidacloprid, in single exposure and in mixture of both insecticides. Body length measurement and estimation of egg production were used as input for the calibration of a DEBkiss-TKTD model with the single exposures. The calibrated model was then used to predict the effects of the mixture, and these simulations compared with the observed effects. Results showed that the *E. crypticus* life cycle under chemical stress can be accurately simulated with a DEB-TKTD model. The physiological modes of action (pMoA) for cypermethrin and imidacloprid were an increase of the energy costs for growth, and an increase of maintenance cost, respectively. The DEB-TKTD model, calibrated with the single exposures, provided an accurate prediction of the mixture effects with r² values (predicted vs observed) of 0.95 and 0.96 for the body length and cumulative egg production. This study, with the development of the combined experimental and DEB-TKTD workflow, supports the robustness and reliability of DEB-TKTD models in their use in pesticide risk assessment.

4.05.P-We189 A User-Friendly Software Tool for Deb-Tktd Model Predictions

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Mechanistic effect models have a great potential to contribute more realism to the environmental risk assessment (ERA) of pesticides. In this context, toxicokinetic-toxicodynamic (TKTD) models have received particular attention and are now available for different applications. TKTD models based on dynamic energy budget theory (DEB-TKTD) have been developed to simulate sublethal effects, exploring the effects of toxicants on growth and reproduction over time. The EFSA scientific opinion on TKTD models for aquatic organisms (EFSA PPR 2018) recognises the great potential of DEB-TKTD models for a future use in prospective ERA for pesticides. However, the scientific opinion (EFSA PPR 2018) also concludes that these models are not yet ready for use. One main obstacle mentioned is the fact that no user-friendly DEB-TKTD modelling tools are currently available. To facilitate the use of DEB-TKTD models for ERA we are developing a user-friendly R-shiny application allowing users to conduct forward projections of defined DEB-TKTD models. Specifically, the tool will be based on the simplified DEB-TKTD models described by Jager (2020). The tool will allow calculating predictions for different exposure profiles and user-provided input parameter sets. It predicts the multiplication factor that would need to be applied to a given exposure profile in order for growth or reproduction to be reduced by X% (termed EP_x by EFSA PPR 2018). The results are then directly compiled in a basic report document. The publication of the software tool is envisaged for mid-2022. It will be freely available and comes along with a user manual. The code will be open source. We present the design and layout of the application and provides an overview of its functionality. References EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2018. Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms. EFSA Journal 2018;16(8):5377, 188 pp. <https://doi.org/10.2903/j.efsa.2018.5377>. Jager, T., 2020. Revisiting simplified DEBtox models for analysing ecotoxicity data. *Ecol. Model.* **416**, 108904, <https://doi.org/10.1016/j.ecolmodel.2019.108904>.

4.05.P-We190 Case Study for Mammal Risk Assessment of Pesticides Using the Deb-Tktd Model

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The draft update to the EFSA guidance for bird and mammal risk assessment (EFSA 2021) emphasizes the great potential of toxicokinetic-toxicodynamic dynamic energy budget models (DEB-TKTD), for example for increasing realism of the risk assessment and reducing the use of test animals (EFSA 2021). Nevertheless, EFSA highlights the lack of published examples of DEB-TKTD modelling for mammals, especially in the assessment of reproductive effects. This lack of examples is currently seen as the major hurdle in the regulatory acceptance of these modelling approaches (EFSA 2021). We present a case study for the use of DEB-TKTD modelling in mammal risk assessment of insecticides. Starting from the published Add-My-Pet DEB model for the Wistar rat (Rakel et al. 2019), we adapted the model to describe the available toxicity data for an insecticide. We extended the existing DEB model (Rakel et al. 2019) to allow for an improved prediction of offspring body weight. Next, we added a TKTD module to the model and calibrated it to the available rat toxicity data, while holding out studies for model validation. Thus, this case study constitutes an example of the successful integration of a variety of studies into a single TKTD model parameterization, allowing for the prediction of realistic exposure scenarios and single endpoints that integrate the underlying toxicity data. References EFSA, Working Group on Birds and Mammals. 2021. *Risk Assessment for Birds and Mammals*. DOI: 10.2903/j.efsa.201Y Kim Rakel, Andre Gergs, Nina Marn, Dina Lika. 2019. *AmP Rattus norvegicus Wistar*. version 2019/12/12

4.05.P-We191 Why a Lemna TKTD Model Can Also Be Applied to Sediment Rooted Macrophytes

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In the Scientific Opinion on TKTD modelling, the EFSA PPR panel (2018) has evaluated also models for primary producers and considered the *Lemna* model of Schmitt et al. (2013) as ready to use in risk assessment. A more complex model for *Myriophyllum spicatum* was found to need further refinements and testing. In this presentation we will show that the basic principles of the *Lemna* TKTD model can be applied not only to floating species like *Lemna* but also to model growth inhibition tests with sediment rooted macrophytes. The original TKTD model for *Lemna* uses six model parameters. Permeability through the cuticle, a plant-water partitioning coefficient to calculate the internal unbound concentration and a metabolisation rate describe the toxicokinetics while EC₅₀, a slope parameter and a maximum effect determine the response to the internal unbound concentration (toxicodynamics). However, a working group of the SETAC IG on effect modelling has recently evaluated the *Lemna* model and concluded that in most cases, the TKTD model can be simplified to a 3 parameter model (with permeability, EC₅₀ and slope). Despite that rooted macrophytes are physiologically different from small floating plants as *Lemna*, we argue that such a simple model is still applicable. Its complexity is similar to the one of the reduced GUTS model to describe lethal effect on e.g. invertebrates and fish. The usually slower growth of rooted macrophytes is just a matter of model parameterisation but not model structure. Growth inhibition tests with macrophytes aim to achieve exponential growth of the control plants and thus, this parameter can directly be taken from the observed control growth. If uptake from sediment pore water instead open water is the most relevant exposure path, the model allows to use measured pore water concentrations as the exposure measure. Modelling both paths simultaneously is not included yet. The model needs some species specific conversion factors between fresh and dry weight, volume and surface area. In addition, for rooted macrophytes the ratio of total shoot length (e.g. for *Myriophyllum spicatum*) or total leaf length (e.g. for *Glyceria maxima*) to dry weight instead of dry weight per frond are needed but are available from the usual tests. In a second poster we will give an example on the application of the model to *Myriophyllum spicatum* including calibration, validation and prediction.

4.05.P-We192 Virtual Refined Exposure Tests With Macrophytes - Using a Generic TKTD Growth Model to Predict the Effects of Time Variable Exposure on Myriophyllum spicatum

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The use of herbicides can result in the exposure of macrophytes in edge-of-field water bodies. Depending on the properties of the active substance, the type of water body, and the soil and weather conditions, exposure models like FOCUS Step 3 predict a variety of time series of the environmental concentrations. Exposure events are often expected to be very dynamic, especially in ditches and streams. This can be addressed by refined exposure tests in contrast to the standard test with intended constant exposure. TKTD models in connection with such tests allow to address a large number of exposure profiles resulting from different uses of the plant protection product and the set of FOCUS scenarios. For the example of an ALS-inhibiting herbicide we tested whether a simple TKTD model already used for the duckweed *Lemna* can also describe the effects of dynamic exposure on the sediment rooted plant *Myriophyllum spicatum*, another standard test species. The model calculates a scaled internal concentration from the concentration in the medium and the permeability of the cuticle determining uptake and elimination. The inhibition of the growth rate is described by a 2-parameter log-logistic function of the scaled internal concentration. Thus, as for the GUTS model for lethal effects, three TKTD parameters have to be calibrated by means of growth inhibition tests. For this case study, the model could be calibrated very well by means of data from a standard test with constant exposure over 14 d and a test with short-term exposure. The model was highly conservative for the results obtained in two other refined exposure tests where the observed effects were smaller than to be expected. Thus, the model was considered suitable for conservative predictions of the effects of dynamic exposure of the herbicide on *Myriophyllum spicatum*. Therefore, the PECs of the worst-case 14 d time windows of FOCUS Step 3 exposure profiles were used as model inputs to simulate standard growth inhibition tests with time variable exposure. According to EFSA's Scientific Opinion on TKTD modelling, EP50, the multiplication factors applied to the FOCUS Step 3 PEC values which result in 50 % inhibition of the growth rate over the 14 d standard test duration (i.e. the Tier 1 assessment endpoint), will be presented.

4.05.P-We193 Applying Aquatic Ecosystem Models to Pesticide Risk Assessment: Data Needs and Lessons Learned

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Aquatic Systems Models (ASMs) are useful for extrapolating the organism-level effects of pesticides observed in laboratory toxicity tests to field-population, ecosystem, and landscape levels, thereby enhancing the ecological relevance of risk assessments. At the same time, aquatic mesocosms provide a complementary empirical approach for higher-tier risk assessments. Here, we address how these modelling and empirical approaches can complement each other in two ways. First, each can provide an independent line of evidence in a weight-of-evidence risk assessment framework. Second, ASMs can extend the value of data from mesocosm experiments by simulating their dynamic biological communities and physical-chemical environments under control conditions, then simulating scenarios corresponding to conditions untested in the empirical systems including series of multi-season exposures with time-variable pesticide concentrations. Lessons learned from our attempts to model pesticide effects in experimental aquatic mesocosms fall into several categories: 1) The availability of data and need to resolve potential mismatches between data generated by mesocosm experiments (e.g., cells/L or Chlorophyll-a for phytoplankton, and counts/sample of macroinvertebrates for some mesocosms) and the data input needs of an ASM (e.g., biomass of phytoplankton/L and biomass of macroinvertebrates/m² for some ASMs); 2) The appropriate level of taxonomic resolution in the models, and whether species or functional groups are modelled; 3) Calibration and validation approaches, especially the importance of Pattern Oriented Modelling and sensitivity analysis, and how to address substantial variability among replicate mesocosms; 4) How to handle lack of immigration/emigration data for some taxa in mesocosms; and 6) The importance of using standardized exposure scenarios and specific exposure concentrations from transport-fate modelling in regulatory programs (e.g., FOCUS profiles). Following the modelling cycle described by others, we recommend explicit definition of Data Quality Objectives for models (What questions are being asked? What data are available? What level of certainty is needed?); early communication between mesocosm study team and modelling team; and explicit characterization of key receptors and food web structure including how those relate to natural systems in agricultural landscapes of interest.

4.05.P-We194 Using a Dynamic Energy Budget Model to Unravel the Physiological Mode of Action of Lead (Pb) to *Lymnaea stagnalis*

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Lymnaea stagnalis is an especially sensitive species for a variety of metals, particularly lead (Pb). However, the observed sensitivity of *L. stagnalis* to lead (Pb) has been highly variable between studies. The exact mechanism of Pb toxicity to *L. stagnalis* currently remains unclear. Under dynamic energy budget (DEB) theory, different physiological modes of action (PMoAs) result in the emergence of distinct changes to the life histories of exposed organisms. DEB models provide a mathematical framework connecting chemical stress at the physiological level to individual-level effects. This work aims to better understand the PMoA of Pb toxicity to *L. stagnalis* by applying DEB modeling to previously published datasets. Two focal studies were selected in order to examine the impacts of Pb exposure on *L. stagnalis*. While initial difficulty in fitting the model to control data in one study led to a partial reparameterization of the model, satisfactory predictions of both growth and reproduction were ultimately achieved. Subsequently, the model (as calibrated to each of the two focal studies) was utilized to evaluate the relative likelihood of several PMoAs. Assuming decreased assimilation, the *L. stagnalis* DEB model was able to capture a variety of

trends in experimentally observed endpoints, including growth, reproduction, and food ingestion. Despite some difficulties experienced with calibrating the model to control data, the weight-of-evidence suggests that decreased assimilation via a decrease in food ingestion is the most plausible PMoA for chronic Pb toxicity in *L. stagnalis*.

4.05.P-We195 A Review of Modelling Approaches for Ecological Risk Assessment of Pesticides

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A wide diversity of pesticides are used for crop protection leading to the contamination of soil, water, and air, and can therefore have ecotoxicological effects on living organisms. It is inconceivable to study effects of each compound on each model species from each ecosystem compartment, experimental studies being time consuming and cost prohibitive, and animal testing having to be avoided. Therefore, numerous models have been developed to assess ecotoxicological effects of pesticides. In this context, our objective was to review modelling approaches enabling the assessment of the effects and the risk of pesticides, including biocontrol substances, on organisms, biodiversity, and ecosystems services. Several model categories were inventoried: QSAR, dose-response, TKTD, SSD, food web, population, community, landscape, and mixture models. These models were developed for various species (terrestrial and aquatic vertebrates and invertebrates, aerial organisms, terrestrial and aquatic primary producers, micro-organisms) and environmental compartments (soil, freshwater, seawater, air). These models are increasingly recognised for the regulatory risk assessment of pesticides but remain rarely used by regulatory bodies. The main limits of these models (for example, long-term effects and functional responses are hardly considered) will be discussed together with improvement avenues (multi-generational effects, multiple biotic and abiotic stressors...). This review will also underline that we are facing a lack of models tested from field data and a lack of sensitivity and uncertainty analyses. Our conclusion will be that accurate modelling of the effects of pesticides and other stressors on living organisms, from their application in the field to their functional consequences on the ecosystems at different scales of time and space, would help going towards a more sustainable management of natural resources.

4.05.P-We196 Scenario Development for Bee Risk Assessment and Health Modelling

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Bee effect modelling has become a core instrument in bee risk assessment (EFSA 2013 'Guidance Document on the risk assessment (RA) of plant protection products (PPP) on bees', EFSA 2018 'A systems-based approach to the environmental RA of multiple stressors in honey bees', EFSA 2021 'Outline of the revision of the Guidance on the RA of PPPs on bees'). Pesticide RA using such models is based on scenarios. In this project we (i) identified key conceptual elements of scenarios in regulatory RA, (ii) developed proposals for these elements, and (iii) generated scenarios for the BEEHAVE model to demonstrate these elements. The example assumed a RA for honeybees related to the use of a PPP in apples located in France. As apple cultivation density increases so does attractiveness of sites for honeybee keeping (occurrence) and exposure potential. Therefore scenario representativeness (level of conservatism) was mainly driven by regional apple cultivation density. Three regions were selected located in different climatic regions to account for weather variability. In these regions, local site selection (i.e., placing the beehive) was done in a combination of bee forage mapping at medium resolution and beekeeper preference. For each site, scenarios were constructed for a 9 km radius around the beehive. In view of a scenario development framework, we propose a tiered scenario development scheme to implement a well-defined level of complexity together with a targeted certainty evaluation. We developed structured bee forage information layers for transparent bee forage modelling: (i) Land use/cover, (ii) Vegetation, (iii) BeeForage. All information layers are spatially and temporally explicit. The transition from one information layer to the next was done by explicit modules, e.g., the BeeForage module represents an approach on how nectar and pollen provision is modelled for a given vegetation and its phenology. According to the tiered scenario scheme, simple modules can be replaced with more sophisticated ones if needed and available. In our example we start with simple representations of processes, e.g., bee forage (nectar and pollen) provision is modelled in five categories (0-4, 4=mass forage) represented in a lookup table defined from literature, by vegetation patches with a monthly resolution vegetation phenology. Due to current technical limitations of BEEHAVE, the spatiotemporally explicit BeeForage(x,t) information is aggregated into < 500 units.

4.05.P-We197 Connecting Heterogeneous Ecological Studies and Their Uncertainties Within a Modelling Framework for Ecological Risk Assessment

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Embedding laboratory or mesocosm studies within monitored landscape in order to assess the real impact of stressors at the ecosystem scale is a huge challenge for ecotoxicological modelling. Assessing the ecological risk of chemical compounds is critical to avoid the decline and potential extinction of some populations that may subsequently threaten other species for example in food chains. Simultaneously, being able to control population dynamics is also important when a population outbreak in specific time and space may compromise agricultural activities and human health. From marketing authorization of chemical products to control strategies, Ecological Risk Assessment studies have to deal with multiple stressors within multiple application contexts. In this talk, we propose a unified modelling framework for ecotoxicology, agroecology and eco-epidemiology. We used this framework under several different contexts and we will present results for two of them: (i) the first one focusing on Bt-maize impact on Non-Target Lepidoptera in Catalonia (Spain), and (ii) a second one on the outbreak of Fall Armyworm resistant population at the continental scale of Australia. We also performed a sensitivity analysis in both cases to identify the most

influential processes and provide recommendations toward ecosystem management practices. Our results illustrate that an appropriate and refined sensitivity analysis may help in identifying general trends in ecological parameters that have influence on population dynamics. Consequently, it can be assumed that an overall sensitivity analysis based on massive computing provides effective insights on the order of magnitude (e.g., the distance at risk to the nearest Bt-maize crop field) and allows to identify the most influential input parameters and variables to consider (e.g., influence of treatments on resistance outbreak depending on their Mode Of Action).

4.05.P-We198 Predicting Saltwater Toxicity From Freshwater Toxicity Using Species Sensitivity Distributions

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In ecological risk assessment, species sensitivity distributions (SSDs) have played a crucial role. Estimating SSDs requires toxicity data for many species, but reports on saltwater species are often more limited than for freshwater species. This limitation can be a barrier to discussing the management of saltwater quality for protecting marine ecosystems, and the relationships between freshwater and saltwater SSDs have been investigated to consider the possibility of extrapolation. In order to determine how accurately saltwater toxicity could be estimated from freshwater toxicity test data, the present study aimed to examine the relationships between the parameters (i.e., mean and standard deviation (SD)) of freshwater and saltwater log-normal SSDs. Freshwater and saltwater SSDs for 104 chemicals were estimated with reported acute toxicity data, and their means, SDs, and hazardous concentrations for 5% of the species (HC5) were compared. As a result, standard major axis regression analyses generally showed that there were nearly 1:1 relationships between freshwater and saltwater SSD means, SDs, and HC5s. The squared correlation coefficients for the SSD means and HC5 values were high, and the ratios of freshwater-to-saltwater SSD means and HC5 values for most of the 104 chemicals fell within the range 0.1–10. Regarding SSD SDs, differences in values between freshwater and saltwater SSDs were relatively small though a strong correlation was not found. These results indicate that a saltwater SSD for a chemical can be reasonably estimated from the corresponding freshwater SSD, when the available ecotoxicity data for saltwater is not available or limited. In addition, differences in the means and SDs between freshwater and saltwater SSDs were larger when the number of test species used for SSD estimation was lower, which suggests that it will be a key to securing enough toxicity data for the prediction. Continuous data collection is required to capture the relationships between freshwater and saltwater SSDs for taking the best advantage of the freshwater toxicity data in saltwater ecological risk assessment.

4.05.P-We199 Applying a DEB Model to Multi-Stressor Exposures of Polyethylene Particles and Sodium Dodecyl Sulphate in *Daphnia magna*

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Daphnia magna are a well-established test organism in toxicity studies and regulatory testing. In addition to the acute 48-hour toxicity test (OECD 202) there is the 21-day chronic reproductive test (OECD 211) encompassing monitoring of growth (often measured as eye-tail length) and variations in reproduction (such as total offspring and time to first and subsequent broods). These test endpoints provide a good starting point for data collection and observations required for the application of a dynamic energy budget (DEB) model. A DEB model describes the rates at which an organism assimilates and utilises energy for maintenance, growth and reproduction, as a function of the state of the organism and of its environment. Data from chronic reproduction tests with *D. magna* exposed to a sublethal dose (25 mg/L) of spherical polyethylene particles (1-4 µm) was used in a BYOM DEB-KISS model to explore potential changes to the daphniid's energy budget in response to a particle-based toxicant. *Daphnia* were cultured and exposed in a high hardness salt medium (HH Combo) with the algae *Chlorella vulgaris* supplied as a daily food ration at *ad libitum* concentrations throughout the test. In addition to this, chronic data of a widely used surfactant, Sodium Dodecyl Sulphate (SDS), was used to establish a baseline response to a chemical based exposure in order to compare the variation in the energy budget due to the differing exposure routes and toxicant interactions i.e. adsorption compared to an ingestion-based pathway. The combined effects of a multi-stressor exposure of *D. magna* to SDS in combination with the polyethylene particles was also used to look at potential changes to the mode of toxicity within the DEB model based on the combined stress and exposure pathways. The DEB model outputs are then discussed in terms of the links to other sublethal indicators of stress, such as changes to the excreted proteins, variations in the concentration and distribution of lipid deposits, and morphological defects in the daphniids over the 21-day testing period, to further explore the links between these sublethal markers and toxicant impacts on the energy deviations from the controls. This raises interesting discussion points associated with the differences in toxicant responses induced in Daphniids' exposed to microplastic particles when compared to a chemical based toxicant response, and to the combined exposure response.

4.06 Environmental assessment and registration of polymers: scientific issues and implementation challenges

4.06.T-01 Improving Available Guidance for Persistence Assessment of Substances (CEFIC-LRI ECO52) - Framework for Polymer Persistence Assessment

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Chemical persistence plays a key role in the determination of environmental exposure making it an important component in risk assessment and regulation. However, existing frameworks under which persistence is assessed have shown some limitations. Chemical degradation rates are prone to wide variability depending on environmental conditions, which is difficult to address in a

weight of evidence approach. In addition, many substances are problematic, or fall outside the applicability domain of existing frameworks due to their specific characteristics. Finally, evaluating degradation half-lives using a compartment-by-compartment approach is overly simplistic, and neglects dynamic multimedia exchanges and degradation processes that may have an important bearing on the overall persistence of a substance in the environment. This CEFIC-LRI project aims to address these issues by improving available guidance for persistence assessment of substances, including those considered problematic to evaluate under the current frameworks. Is the concept for persistence assessment of substances applicable to plastic articles, for which persistence can be a beneficial property? As natural polymers are usually not regarded as a concern under real environmental conditions, better understanding is necessary to improve our methodology of polymer persistence assessment and to provide guidance to assess man-made polymers. The project aims to give information on essential elements to justify persistence assessment of polymers in a weight of evidence, and to give suggestions for future needs and research. Available literature was reviewed for polymer specific methodologies to identify influencing testing parameters, knowledge gaps and areas of improvement. Four major challenges have been identified for assessing polymer biodegradation by Albright and Chai (2021). Challenges have also been discussed in the ECETOC technical report No. 133-2, and a general outline for polymer (bio)degradation assessment has been presented. An overview and discussion is given about challenges of polymers with respect to the standard assessment scheme, and about suitable alternatives. A combination of all approaches seems to be most appropriate. Finally, a framework (workflow) is presented which includes the essential elements such as profiling of the polymer, scientific differences between natural and synthetic polymers, and on weathering for example by sunlight (UV) for the weight of evidence.

4.06.T-02 Modelling Cationic Polymers Aquatic Toxicity

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This presentation summarizes the CEFIC LRI study (ECO46 – iTAP) on how to improve the aquatic risk assessment of cationic polymers. The amount of publicly available, high quality environmental toxicity data on industrial polymers such as cationic polyquaterniums is however extremely limited. Given the large size of the materials traditional hydrophobicity driven toxicity is not expected. Other relevant descriptors for cationic polymers thus need to be identified. Molecular weight and charge density are well-known physical-chemical attributes that are suspected to be correlated with aquatic toxicity. Cationic polymers are polymers of potential environmental concern. They can be based on quaternary ammonium (PQ) used in personal care and household cleaning products as conditioners or softeners, and as flocculants in drinking water treatment plants. Developing (Q)SARs based on curated cationic data is challenging as the data availability, transparency and quality for the training set is limited and insufficient polymer descriptor information is available. We developed multivariate models for cationic polymers in accordance with the five principles of the *Guidance Document on the Validation of (Q)SAR Models* (OECD, 2007). Data was provided by Environment and Climate Change Canada (n = 242) and the USEPA (n = 73). The data was blinded in terms of full structural disclosure but contains information on key structural features allowing an analysis of the relative contribution of these to the overall toxicity. The data consisted of two fish species (*P. promelas* and *O. mykiss*), and green algae (*S. capricornutum/P. subcapitata*). It has been suggested by Boethling and Nabholz (1997) in that the aquatic toxicity of cationic polymers is primarily (solely) governed by the charge density. The table below reflects our results of a multivariate analysis of the contribution features of the compound have on the toxicity towards fish and green algae. The area of (Q)SAR modeling for the evaluation of toxicity of polymers has remained largely unexplored. Our study revealed that the presence of a cationic functional group in the pendant chain at positions; one, four and five enhances the toxicity. Similarly, higher charge density results in an increase in the toxicity towards fish and green algae and higher average molecular weight results in higher toxicity towards green algae. Presence of primary and secondary amines in the molecular building block result in a reduction in the toxicity, while the presence of quaternary amines in the molecular structures results in an increase in toxicity against green algae. Further work is needed to fully elucidate the toxicity contributions and validate regulatory applicable models for defined classes of polymers. Moreover, further work is needed to clarify the regulatory acceptance of multivariate models, as well as interspecies models for gap filling, as univariate models may not accurately predict toxicity as multiple descriptors affect the overall toxicity in addition to the charge density.

4.06.T-03 The ECETOC Conceptual Framework for Polymer Risk Assessment (CF4Polymers): Considerations & Examples for Grouping of Polymers

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The building block nature of polymer chemistry and the corresponding definition of polymers in several jurisdiction globally demand for a targeted and dedicated strategy for polymer grouping when attempting for a systematic polymer evaluation under a

chemical risk assessment framework. Related polymers are often homologues manufactured from the same starting materials and similar processes, leading to a large number of similar structures. But even if few of the building blocks are different, such chemical variation in a small part of a macromolecule does usually not lead to differences in physico-chemical or biological properties. In the present contribution, the internationally agreed grouping concepts has been further advanced to better scope the complexity and versatility of polymers. Definitions going beyond the internationally agreed grouping concept have been introduced in the ECETOC Task Force report 133-3. A central role has been assigned to the term 'hazard similarity' that forms a central element of the grouping of polymers. It is the overarching aim of the polymer grouping approach to define 'hazard similarity' of different polymers and, consequently, the final group. Generally, polymer groups based on similar hazards can be expected to contain many more members than categories for non-polymer substances. The reason is the building block nature of polymer chemistry. Specifically, similarity is based upon three criteria to determine and to justify similarity: (Criterion 1) chemical nature; (Criterion 2) physico-chemical property/ies; and most importantly (Criterion 3). All three Criteria correspond with each other, and they are critical for the full description of the polymer group. The properties of each criterion also define the content and the boundary of the group. If the defined group is understood as one 'substance' (in a regulatory context), the content and boundaries of the three Criteria can serve as descriptors for substance identity. The polymer grouping approach was exemplified in three case studies, i.e. on BADGE polymers, Polyetheroles, and on surfactant polymers. The polymer grouping approach proposed here allows to significantly simplify and structure the data requirements for polymer hazard and risk assessment and gives rise to a pragmatic and reasonable description of the substance identity for polymers based on the newly introduced principle of hazard similarity.

4.06.T-04 Putting the ECETOC Conceptual Framework for Polymer Risk Assessment (CF4Polymers) Into Practice: Case Studies on Cationic Polymers

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Polymers are currently being reconsidered in the context of regulatory programmes, and this raises a number of technical and scientific challenges as polymers represent a diverse chemical space and are quite different from discrete mono-constituent substances. Against this background, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) decided that a review of relevant scientific methods and knowledge applicable to the risk assessment of polymers would be helpful to provide a scientific perspective for the safety assessment of polymers. As an outcome of its work, the ECETOC Polymers TF has been preparing the ECETOC Technical Report (TR) No.133 series, of which this report is the third and final presents *Case Studies Putting the ECETOC CF4Polymers into practice*. The case study on cationic polymer is one of the seven case studies selected from the diverse universe of polymers to further evaluate (1) the usefulness of the CF4Polymers (ECETOC TR No. 133-1) for the safety assessment of different types of polymers and (2) the information on the applicability of tools, methods and models for the hazard and risk assessment of polymers presented ECETOC TR No. 133-2. It is important to make clear that the case studies were not intended to document a comprehensive risk assessment for any specific polymer. Rather, publicly available data and unpublished TF company data were collated and assigned to the eight steps of the CF4Polymers presented in ECETOC TR No. 133-1 to evaluate the scientific usefulness and comprehensiveness of the process through use of examples. The case study on cationic polymers served to illustrate how the CF4Polymers *can be used* for polymer hazard and risk assessment. Further, the collated data were used to assess the applicability of tools, methods and models for polymers in environmental and human health risk assessment.

4.06 Environmental assessment and registration of polymers: scientific issues and implementation challenges (Virtual Only)

4.06.V-01 Algal Toxicity of Cationic Polyquaternium Polymers

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Cationic polymers, such as polyquaterniums (PQ), are widely used in water purification processes, wastewater treatment, and in personal care products. The flocculating ability of these materials is often leveraged to remove suspended solids from mining effluent or wastewater. It is therefore not surprising that algal clumping has been observed in some, but not all, cationic polymer standard toxicity tests. In this study, standard 72h OECD 201 algal toxicity tests with *Raphnidocelis subcapitata* were completed using several representative PQ materials (PQ6, PQ10, PQ16). Test materials were chosen to encompass a range of molecular weights and charge densities to determine the influence of test material characteristics on toxicity. Tests were conducted in two different laboratories to quantify inter-laboratory variation. Algal clumping was observed in some, but not all, polyquaterniums and was inconsistent within exposure concentrations. All concentrations were gently homogenized daily in order to improve growth estimation and repeatability of the assay. PQ6 toxicity was consistent between labs and across PQ6 materials with different molecular weights. Toxicity for PQ6 and PQ16 materials were correlated with charge density. This aligns with previous observations in the literature. Very steep dose response curves and low acute to chronic ratios (< 2) were observed for PQ6 and PQ16 materials, suggesting a physical MOA. PQ10 was several orders of magnitude less toxic than PQ6 and PQ16. Flat dose-response curves and significant clumping was observed. Mitigating factors like addition of organic carbon in the form of humic acid will also be presented.

4.06.V-02 Application of Standard Screening Biodegradation Tests to Water Soluble Polymers

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The objectives of this research were to evaluate the biodegradation of a suite of water-soluble polyethylene glycol (PEG) materials ranging in average molecular weight (MW) from 4000-500,000 using activated sludge and river water inocula. The tests followed the OECD 301B, OECD 302B and OECD 301F guidelines. We wanted to probe the ability of regulatory accepted screening test methods to evaluate the biodegradability of water-soluble polymers with a wide range of MWs and use inocula from different environmental compartments. Further we were interested in gaining understanding of the time necessary for complete mineralization in these types of test systems for a range of polymer MWs. Additionally, we gained insight into test system viability and sensor stability as tests were extended well beyond traditional test durations. Finally, we wanted to evaluate the impact of the polymer endcap (due the changes in synthesis route from PEG to PEO) on biodegradability of polymers with similar MWs.

4.06.V-03 Cationic Polyquaternium Acute Toxicity and Toxicity Mitigation in *D. Magna*: Implications for Chronic Toxicity Testing and the ACR

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Cationic polymers, such as polyquaterniums (PQs), are often used in shampoos and conditioners because of their anti-static properties. Cationic polymers have been shown induce toxicity to aquatic organisms, and these compounds have been flagged by chemical regulators as potential “polymers of concern” that are likely to receive additional regulatory scrutiny in the future. Cationic polymer toxicity has been shown to vary based on polymer physical/chemical properties, and previous studies have also shown that cationic polymer toxicity can be ameliorated in the presence of dissolved organic carbon. This toxicity mitigation may also impact chronic invertebrate testing, as chronic toxicity tests are done in the presence of food (algae). Therefore, this research first explores acute PQ toxicity to *Daphnia magna* as it relates to molecular weight (MW) and charge density (CD), followed by an investigation of PQ toxicity mitigation by humic acid (HA). The chronic toxicity of a subset of PQs was also conducted, coupled with an investigation of acute toxicity in the presence of food to explore how the addition of food may impact the toxicity outcome. Results of this work indicate that PQ toxicity is correlated with cationic CD but not with MW, and that PQ toxicity can be mitigated by environmentally relevant concentrations of HA. The presence of food in acute toxicity assays was also found to alter PQ toxicity. This work reveals that, due to the sensitivity of PQ toxicity to water quality parameters (e. g. TOC), special considerations should be given when applying standard toxicity extrapolation methods, like the acute to chronic ratio, for predicting chronic toxicity. More work is needed to characterize PQ toxicity mitigation relative to polymer characteristics and water quality parameters to aide in more accurate environmental hazard assessments of these materials.

4.06.V-04 Comparative Toxicity Assessment of 16 Different Polymers in *Daphnia magna*

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Polymer materials are currently exempted from registration under REACH due to low concern for toxicity based on the high molecular weight (ECHA April 2012). However, with the increasing attention to the pollution of the environment with persistent plastic debris, research on the effects of microplastics in particular on environmental organisms intensified. The study presented here aims at adding to the knowledge on polymer toxicity by including various modifications of 6 different polymers and assessing their influence on *Daphnia magna* immobilisation. In total, micro-sized particles in 15 variations of TPU, PU, PMMA, LDPE, PE and PA were subjected to testing in the 48 h acute immobilisation assay, including a tire rubber particle positive control. For all polymer particles, dispersal in daphnia medium was facilitated by the addition of TWEEN-40. Non-toxicity of TWEEN-40 in the respective concentration was confirmed by pre-tests. The characteristics of the polymer particles differed not only in polymer type, but also with regard to size distribution, aromaticities, and crosslinking degree. All microplastic particles were tested in concentrations from 10 µg/L up to 1 g/L. Uptake of particles in the gut was assessed by microscopic inspection. None of the tested polymer microparticles exerted toxicity towards *Daphnia magna* during the time of observation. For some exposures, an attachment of particles to the carapaces (both before and after moulting and the antenna was observed. All tested microplastic particles were taken up by the organisms as visible by light or dark (in the case of tire rubber) coloration of the gut. Excretion of particles was observed as well. Surprisingly, also in the TWEEN-40 only exposures, a light coloration of the gut resembling those of particle exposed organisms was visible. It was confirmed by particle-only exposure of one selected polymer that uptake occurs also without the TWEEN-40 present, and that the coloration is indeed comparable. Overall, the results of our study demonstrate that no acute toxicity occurs irrespective of the various particle properties and irrespective of particle uptake and attachment to organisms. Neither particle size nor polymer type or the different polymer end groups had an influence on toxicity. Reference ECHA (April 2012). “Guidance for monomers and polymers Version 2.0, Guidance for the implementation of REACH.” [European Chemicals Agency ECHA-12-G-02-EN](#).

4.06.V-05 Understanding Ecotoxicological Responses of Fish Embryos to Cationic Polymers

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Cationic polymers have been highlighted as potential polymers of concern. Regulatory interest in polymers has been constrained on the assumption that polymer size will result in an inability to pass biological membranes and will therefore be likely to have limited interaction with man and the environment. In this presentation we describe a series of fish embryo toxicity (FET) assays used to establish a baseline understanding of several representative polyquaternium categories (PQ6, PQ10, PQ16). Materials were chosen to encompass a range of molecular weights and charge densities to determine the influence of test material characteristics on toxicity. Due to the ability for organic carbon to influence fish response to cationic polymers humic acid toxicity mitigation was also explored. Chorionated and dechorionated FET tests were comparable (z-test, $p=0.8962$). 96 h LC50 values for the four PQ-6 Merquat compounds, with charge densities of 6.2 mEq/g, ranged from 0.35 to 0.92 mg/L for chorionated and dechorionated tests which are comparable to the mean OECD FET validation PQ-6 Merquat 100 data. Existing SAR models for cationic polymers with carbon-based backbones attribute differences in polymer charge density. Toxicity in chorionated and dechorionated FET tests was correlated with charge density ($R^2=0.89$ and 0.94 , respectively), with the relationship following the same curve shape as the data from Boethling and Nabholz ($R^2=0.73$). Toxicity amelioration in the FET varied with HA source and concentration and with cationic polymer and concentration. Testing included dilution water controls, HA controls, and polymer concentrations at or above the 96 h LC50 values with and without HA. Amelioration by HA occurred in a dose dependent manner. These studies provide context for future testing of cationic polymers in an environmental risk assessment context. Characterization of polymers is of extremely high importance and needs exist for method standardization and regulatory prioritization. Production lots vary and chemicals given the same CASNO clearly can possess an array of potencies. Cationic polymer charge density clearly plays a role in toxic potency as does general water quality (organic carbon, water hardness, especially). As with test material characterization, the determination of water quality must accompany cationic polymer ecotoxicity data.

4.06 Environmental assessment and registration of polymers: scientific issues and implementation challenges (Poster)

4.06.P-We200 Critical Review of Methods to Determine Surface Tension and Solubility of Polymers

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The CESIO Working Group "Test Methods of Surfactants" (TMS) and the TEGEWA Working Group "Surface Active Substances" have identified the need to look into norms and guidelines concerning surface tension and solubility of surfactants and/or surface-active polymers. The motivation to care about this topic is that assignment of polymers as surface-active should be on account of their properties, but should not be caused by shortcomings of the analytical methodology. For example, the OECD Guideline 115 on surface activity is basically from the pre-computer era; the plate and ring method as described there are real classics (>100 years old!) and suffer from the general problem of ill-defined surface age, which could be especially problematic in case of surface-active polymers. Unfortunately, this is the only guideline on surface tension current (and hopefully not too many future!) regulations can refer to. In addition, it is unclear how the OECD guidelines 105 and 120 on solubility should be applied to surfactants and surface-active polymers, since (A) surfactants do not have a saturation concentration, and (B) surfactants form micelles which scatter light, and this Tyndall effect (i.e. the presence of micelles) – according to the guideline – invalidates the solubility test. So it needs to be discussed whether attaching a fatty acid chain of natural origin to a readily biodegradable water-soluble polymer like PEO should really yield an "insoluble polymer particle". Therefore, there is an urgent need to revisit outdated or unsuitable methods. In the case of surface tension, this should be relatively straight-forward, since today there is already a well established alternative method available: Advances in image processing/computing power during the past two decades have allowed drop shape analysis (Pendant Drop Tensiometry) to develop into a well-established, better alternative to determine surface tensions. Therefore, just the corresponding norm or guideline for the field of surfactants is missing. Respective activities within DIN NMP NA 62-5-63 and CEN TC 276 WG2 have been initiated.

4.06.P-We201 Polymers and Their Toxicity to Algae - Lessons Learned From a Comparative Study

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Due to the different areas of applications, polymers are a very heterogeneous group of chemicals. While the ecotoxicity of nanomaterials has been intensively investigated during the last years, the information on the toxicity of larger particles including polymers is still limited. To improve the knowledge, we investigated the ecotoxicity of eight polymers to algae (*Raphidocelis subcapitata*) in the growth test (OECD TG 201). The polymers differ in their chemical identity, polymer backbone, aromaticity, crosslinking degree, size distribution and dispersibility. None of the materials showed toxic effects. Size as the only reason for non-toxicity can be excluded since small (300 nm) and large materials (267 μm) were taken into account. The same applies for the state of the material, as at least one of the materials was available as a dispersion while others were powders. Furthermore, the surface areas as an influencing factor was discounted as this property differs for the various materials by a factor of 100. Six of the polymers could only be dispersed in the test medium using Tween 40 as dispersant. The use of dispersants in the toxicity testing of chemicals is limited and for nanomaterials it should be avoided. We included this approach to cover the range from no toxicity, due to no presence in water, to potential toxicity in the presence of a dispersant. Further experiments showed that a comparable dispersing effect can be achieved using a concentration of algae and their exudates which is similar to the conditions at the end of the test. We conclude that the missing ecotoxicity in the presence of Tween 40 should not be considered as a false-negative result. Tween 40 simulates a behaviour which can also occur in the environment. The advantage of Tween 40 over natural substances (algae exudates, humic acids) is the defined chemical composition. Heteroagglomeration has been identified as driving factor for

ecotoxicity to algae, at least for nanomaterials. Microscopic images of the test dispersions showed that there was no interaction between polymers and algae which agrees with the observed non-toxicity. Due to the large size and the lack of heteroagglomeration, the probability of polymers being toxic seems to be low, unless they contain bioavailable toxic components.

4.06.P-We202 Towards a Framework for Environmental Fate and Exposure Assessment of Polymers

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There is an emerging need to develop environmental risk assessment methodologies for polymers in order to prevent negative environmental impacts; however, existing environmental risk assessment approaches for low molecular weight chemicals will likely need to be adapted to account for the diversity and complexity of polymers as well as their unique properties. This poster will present key points from a literature review focussing on the challenges and opportunities for the fate and exposure assessment of polymers in the context of regulatory environmental risk assessment of low molecular weight chemicals. This includes a discussion of the applicability and adequacy of existing property and environmental fate parameters for polymers, along with proposed additional parameters which may be necessary to characterise polymer fate, such as attachment efficiency, particle size distribution, and hydrodynamic radius, among others. The implications of these parameters in various stages of an environmental exposure assessment framework will also be presented, with a distinction between solid polymers (such as microplastics) and dissolved polymers (including water-soluble polymers) being key for development of such a framework. Key considerations relating to: polymer identification, grouping and characterisation; polymer degradation and transformation in the environment; development and standardisation of analytical methods for characterisation of polymers and their fate properties; and exposure models for polymers, will also be presented, along with key knowledge gaps and research needs moving forward.

4.06.P-We203 Ecotoxicity and Biodegradability of Water-Soluble Polymers, Used in Cosmetics and Personal Care Products

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Although the research on microplastics is still growing exponentially, another form of plastics, such as water-soluble polymers (WSPs) have been greatly overlooked by scientists, society, and policy makers. They are used in many applications and their annual production in Europe is estimated at several million tonnes. However, their effects and fate in the environment remain unknown. In this context, the aim of our study was to evaluate the ecotoxicity and biodegradability of four WSPs (three of them in solid and one in liquid form) commonly used in cosmetics and personal care products. All four WSPs were polyacrylic acid-based polymers and further characterization revealed that the liquid WSP contained nanoparticles. Ecotoxicity was tested with several organisms, namely aquatic plant *Lemna minor*, microalga *Pseudokirchneriella subcapitata*, crustacean *Daphnia magna*, bacterium *Allivibrio fischeri*, and mixed bacterial culture (heterotrophic and nitrifying microorganisms of activated sludge). All four WSPs had low (10?40%) or moderate (41?60%) effects on the tested organisms at a concentration of 100 mg/L. However, the liquid WSP had a specific toxic effect on *A. fischeri* and nitrifying microorganisms of activated sludge. It caused $73 \pm 1\%$ inhibition of bioluminescence of *A. fischeri* and $88 \pm 17\%$ inhibition of the oxygen consumption by nitrifying microorganisms. The presence of this WSP in the wastewaters could have implications for the nitrification process in wastewater treatment plants. All four tested WSPs were not readily biodegradable, suggesting possible persistence in the natural environment.

4.06.P-We204 Case Studies Putting the ECETOC Conceptual Framework for Polymer Risk Assessment (CF4Polymers) Into Practice

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An ECETOC Task Force entitled 'Assessing the human health and environmental safety of polymers' has been working on the topic of polymer safety assessment since January 2018, and has published three reports (ECETOC, 2019; ECETOC, 2020; ECETOC, 2021). In the third report (ECETOC, 2021) the ECETOC Task Force developed a series of case studies for a range of polymer types and applications. The case studies evaluated: (1) the usefulness of the CF4Polymers presented in ECETOC TR No. 133-1 (ECETOC, 2019) for the safety assessment of different types of polymers; and (2) the information on the applicability of tools, methods and models for the hazard and risk assessment of polymers presented in ECETOC TR No. 133-2 (ECETOC, 2020). The case studies were not intended to document a comprehensive risk assessment for any specific polymer. Rather, publicly available data and unpublished company data were collated and assigned to the eight steps of the CF4Polymers to evaluate the scientific usefulness and comprehensiveness of the process through use of examples. The case studies covered different types of polymers and/or different types of intended uses, and comprised the following: Case Study 1 – polycarboxylates, polyacrylates and polymethacrylates; Case Study 2 – cationic polymers; Case Study 3 – polyolefins; Case Study 4 – solid bisphenol-A

diglycidylether (BADGE) polymers (solid BADGE epoxy resins); Case Study 5 - polyetherols (PEOLs; or polyether polyols); Case Study 6 – surfactant polymers; Case Study 7 – selected professional and consumer uses of polyurethane and polyurea. Overall, the case studies confirmed the value of the CF4Polymers as a consistent framework for risk assessment of polymers whilst providing flexibility for applicability to different types of polymer as there is no “one size fits all” approach, thus highlighting the need for critical case-by-case assessments. Key highlights from each case study will be presented, along with overall learnings across the various case studies. ECETOC. 2019. The ECETOC conceptual framework for polymer risk assessment (CF4Polymers). May 2019. Technical Report No. 133-1. ECETOC. 2020. The applicability of analytical tools, test methods and models for polymer risk assessment. March 2020. Technical Report No. 133-2. ECETOC. 2021. Case Studies Putting the ECETOC Conceptual Framework for Polymer Risk Assessment (CF4Polymers) into Practice. September 2021. Technical Report No. 133-3.

4.06.P-We205 Polymer Registration Under EU REACH: Scientific Issues and Implementation Challenges

Warren Scott, CSI-Europe

Expanding REACH registration requirements to polymers is not as straightforward as it sounds. Due to the complexity of polymer chemistry with its vast array of building blocks and subsequent possible combinations, one size does not fit all in terms of using non-polymer registration data requirements. A more targeted approach is required, but in order to reduce the burden on industry and reduce animal testing, polymers requiring registration will be grouped based on chemical and physico-chemical parameters. This poster will look at the current EU REACH registration procedures for polymers and their monomers, and look ahead at how industry can identify their polymer substances. If grouped as a polymer requiring registration (PRR) substance, what data requirements will they be subject to? Additionally, this poster will look at complications due to Brexit, and how the UK Health & Safety Executive (HSE) plans to tackle polymers under UK REACH.

4.07 Environmental Risk Assessment of Organic and Inorganic UV filters (Virtual Only)

4.07.V-01 Eco-Epidemiology to Assess Potential Risks of Natural and Anthropogenic Factors, Including UV Filters, to Corals in Hawaii

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Recently questions have been raised regarding the environmental safety of some UV filters used in personal, skin care and beach products to corals. In some cases (Hawaii, Key West, Palau) regulatory actions have been precautionary, leading to bans. Eco-epidemiology is a methodology that considers impairments to environmental species and communities from complex combinations of multiple physical and chemical factors with the intent of developing weights of evidence for potential causal relationships. In this study a large set of natural and human influenced factors (including potential risks of measured UV filters and beach visitors) along with coral cover data were assembled to assess the potential adverse effects of UV filters on corals surrounding Oahu, Hawaii within the context of other factors. Principal component analyses simplified the coral data into two components: PC1 representing species diversity and PC2 representing abundance. These components along with all other factors were correlated against each other to determine if some factors could act as proxies for other factors via multiple linear regression and boosted regression tree analyses. Overall, there were good agreements between the two regression methods. The boosted regression tree for PC1 (diversity) showed that 90 percent of the variance were addressed by 3 factors: wave power, temperature (long-term mean) and benthic turf algae. The remaining 10 percent included 13 other factors including beach visitors and UV filters. The regression tree for PC2 (abundance) illustrated 3 factors (temperature long-term mean, latitude, and temperature long-term standard deviation) addressed 75% of the variance. Twelve other factors, including beach visitors and UV filters) were associated with the remaining 25 percent. Hence, it appears that UV filter hazards do not significantly address reduced coral diversity and abundance, suggesting that precautionary bans may not achieve their intended results.

4.07.V-02 Effects of Organic Uv-Filter on Development and Thyroid Endocrine System Using Dre-Mir-499-/- Zebrafish Embryo/Larvae

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Homosalate (HS) and octisalate (OS) are widely used as organic UV filters and have been frequently detected in the water environment. Although their estrogenicity and androgenicity have been reported, the studies on thyroid endocrine disruption are limited. In the present study, the thyroid endocrine potential of HS and OS was investigated using wild-type and dre-miR-499 knockout (dre-miR-499^{-/-}) zebrafish embryo/larvae. Following 96 h exposure to HS and OS (0, 0.003, 0.03, 0.3, 3, 30 and 300 µg/L), embryo coagulation, hatchability, and developmental toxicity were observed. Levels of triiodothyronine (T3) and thyroxine (T4) and transcription of genes related to the hypothalamus-pituitary-thyroid (HPT) axis were measured in wild-type zebrafish exposed to target chemicals. To explain underlying mechanism related to miRNA, we applied dre-miR-499^{-/-} zebrafish and compared the larval mortality with wild-type. Body length and whole-body levels of T3 was tendency to increase. in fish exposed to HS and OS. In fish exposed to HS and OS, significant up-regulation of *tra*, *trfβ*, and *trh* genes and down-regulation of *crh*, *tshβ*, and *deio2* genes were observed. The results of significant decrease of larvae survival in dre-miR-499^{-/-} fish exposed to HS and OS, suggest that dre-miR-499 plays an important role in the toxicity of both chemicals.

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4.07.V-03 Evaluation of Environmental Stressors to Coral in the Florida Keys

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Coral decline has been observed worldwide including to reefs in Florida. Several global and local stressors have been implicated as contributors to the decline of coral populations. The most pervasive and deleterious stressor is the global event of rising atmospheric CO₂ levels which results in sea temperature changes and acidification. These global warming changes cause coral bleaching and ultimately coral death. Stony Coral Tissue Loss Diseases (SCTLD) is causing widespread devastation in the Florida Reef Tract. Expert coral biologists have identified the most important stressors to reefs in Florida – invasive species, unsustainable fishing practices, coastal development, untreated or poorly treated wastewater, urban and agricultural run-off, and tourism-related damage. Recently, questions have been raised about the potential for certain sunscreen active ingredients (UV filters) to contribute to the decline in coral ecological status. An investigation was conducted to evaluate environmental stressors to coral in the Florida Reef Tract with a focus on the Florida Keys through the lens of possible global and local factors. Stressors with potential to impact coral ecosystems in the Florida Keys were identified and prioritized. A weight-of-evidence did not verify sunscreens as a contributor to coral decline. Improving the ecological status of coral in the Florida Keys requires prioritizing efforts on the most significant stressors. Mitigation efforts to restore damaged coral have been successful to some extent but are labor and time intensive. Conservation efforts should focus on recreational practices such as educational efforts on boaters, divers, and other activities in and around the coral reef to reduce the spread of SCTLD and minimize structural damage to the reefs.

4.07 Environmental Risk Assessment of Organic and Inorganic UV filters (Poster Corner)

4.07.PC-Tu01 Freshwater Environmental Safety Assessment of Ethylhexyl Methoxycinnamate in the United States

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Organic ultraviolet (UV) filters are used in cosmetic and personal care products (CPCPs), including sunscreens (considered over-the-counter drugs in the United States), due to their ability to absorb solar radiation. UV filters are released down-the-drain through cleansing, bathing, or rinsing of skin or through the laundering of clothing. The fraction of CPCPs that are washed-off down-the-drain enter wastewater treatment where they are removed to varying extents and discharged to effluent-receiving freshwater. It is therefore important to establish the environmentally safe use of UV filters entering the freshwater compartment. In this presentation, a freshwater environmental safety assessment is conducted for ethylhexyl methoxycinnamate (EHMC) in the United States. The environmental risk assessment (ERA) follows an established framework for CPCPs. For the exposure assessment, the spatially resolved aquatic exposure model, iSTREEM, is used. The iSTREEM model was specifically developed for chemicals disposed of down-the-drain and provides a comprehensive exposure assessment through the consideration of spatial variability in emissions, wastewater treatment, flow, and chemical loads transported from upstream to estimate CPCP concentrations in effluent-receiving freshwaters across the conterminous United States. The model output is a predicted environmental concentration (PEC) distribution representative of the spatial variability in conditions across the region. For the hazard assessment, a review of toxicity data from regulatory databases and the peer-reviewed literature was conducted. Data related to ecologically relevant endpoints for risk assessment (e.g., mortality, growth, reproduction) from studies conducted according to or similarly to standardized test guidelines were selected. From the suitable toxicity data, the most sensitive endpoint was selected to calculate the predicted no-effect concentration (PNEC). The PNEC was calculated by dividing the most sensitive endpoint by the appropriate assessment factor. A safety assessment was conducted by comparing the PEC distribution with the PNEC. The results indicate that freshwater exposure resulting from down-the-drain emissions of EHMC is of low concern as the PNEC does not exceed 90th percentile PEC. These results are instrumental in demonstrating the environmental safety of organic UV filters in the United States freshwater environment and will help prioritize future work.

4.07.PC-Tu02 Developing Standard Ecotoxicity Tests on Scleractinian Corals for UV Filters and Related Chemicals

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Coral reefs are marine hotspots of biodiversity and provide vital ecosystem services. Due to climate change and ocean acidification, reef building corals are declining at a dramatic pace. Not only global but also local stressors impact coral health. UV filters used in personal care products such as sunscreens are considered to be among those substances due to their direct release into the coastal marine environment. Some research findings indicate a possible impact on corals after exposure to certain UV filters such as bleaching or even mortality, whereas other scientific publications did not. Thus, the existing results are considered as controversial due to experimental deficiencies and require further investigation on corals. Using standardised and validated testing protocols would certainly help for a sound and scientifically based conclusion and will also support regulatory decision-making processes. Consequently, we currently develop short and long-term testing methods on corals of different live stages (e.g., larvae and adult stage) suitable for validation and standardisation within the DIN, ISO and/or OECD framework. So far, our

developed ecotoxicity assays for chemicals including those that are poorly water soluble (i.e., UV filters) on corals of different life stages showed reliable test results. As an example, our coral larval settlement assay presented an inverse correlation of the endpoints mortality and metamorphosis, whereas our adult coral bleaching assay displayed a positive correlation of the endpoint bleaching in a concentration-response relationship. As a next step, additional pre-validation work in collaboration with additional laboratories is needed to assess the interlab variability and reproducibility of our testing method and results.

4.07.PC-Tu03 Ecotoxicological Evaluation of Sunscreen Creams on Marine Plankton

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In recent years, a large number of sunscreens have emerged to protect our skin from the damage caused by UV rays. Most of them are made up of simple or compound aromatic structures, sometimes conjugated, which can pose a threat to marine ecosystems. In order to understand their effects on the marine environment, different toxicity bioassays were carried out using two representative zooplankton organisms: sea urchin larvae (*Paracentrotus lividus*) and copepod nauplius larvae (*Acartia* sp.). In addition, a representative of phytoplankton, the microalgae *Tisochrysis lutea*, was chosen. The aim of these tests was to expose these three species with different dilutions of six sunscreen formulations. The endpoints to be evaluated were the larval length at 48 h of *Paracentrotus lividus*, the mortality at 72 h of *Acartia* sp. and growth inhibition at 72 h of *Tisochrysis lutea* cultures. Toxicity was estimated from the NOEC (no observed effect concentration), LOEC (lowest observed effect concentration) and EC₅₀ (median effective concentration). According to our results, none of the creams can be considered totally innocuous for plankton. All of them showed a great variability in toxicity on the different plankton organisms. The highest toxicity level was found in cream number 4 when tested on the sea urchin, exhibiting an EC₅₀ = 74.4 mg/L. In an attempt to identify the component causing the toxicity of cream 4, a UV-filter only present in this cream, 2-phenyl-5-benzimidazolesulfonic acid, was tested on sea urchin. This chemical was found to be more toxic when the sea urchin larvae were incubated with photoperiod 16:8 of light: darkness, obtaining an EC₅₀ = 2.7 g/L and requiring to be diluted 30 times in seawater to lose its toxicity. This study highlights the need to understand the toxic effects generated by solar protection products, as well as their components, on marine organisms.

4.08 Expert Knowledge Elicitation in environmental assessments - subjective, but scientific

4.08.T-01 The Quality of Judgements in Conservation Decision Making

Mark Burgman, CEP, Imperial College, London, United Kingdom

Conservation science is a crisis discipline in which scientists measure impacts in terms of species extinctions and ecosystem collapse, and practitioners typically seek solutions that preempt irreversible change. Present challenges such as global losses of biodiversity and social-ecological systems require efficient and timely action. Decisions need to be made quickly, yet the data and understanding necessary to assess problems and provide unequivocal solutions are typically unavailable, incomplete, dated, or biased. These issues are amplified by a suite surprisingly common procedures and actions that – without malicious intent – misuse or misrepresent data and analyses, generating spurious results and misleading advice. Here, I review the quality and reliability of expert opinion used to fill information gaps and assess the quality of scientific evidence, present some recent empirical results on the limits of ecological judgement, and discuss the prospects for improving expert judgement.

4.08.T-02 Assessing the Calibration of Expert Elicited Probabilities: The Good, the Bad, and the Ugly

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Probabilistic predictions are frequently required in the context of risk analyses and decision making, including ecological risk assessments and human health risk assessments. The need of structured protocols to elicit expert predictions is well established. Such protocols ensure that the elicited judgements (a.k.a. the expert data) can be used as scientific data, since they are subjected to the scientific principles of review, critical appraisal, and repeatability. However, objectively evaluating the expert data, even though essential, is not a trivial task. Experts performance, and hence the quality of the elicited data should be evaluated by scoring experts on questions related to the elicitation questions, whose answers are known a priori, known as seed questions. Experts' performance on seed questions can be measured in terms of accuracy, calibration and informativeness. In this research we are concerned with calibration measures and the interpretation of their exact values. Calibration measures are random variables and their distribution plays an important role in such interpretations. Cooke (1991) proposed a calibration measure for probabilistic predictions (binary variables) that has an asymptotic distribution. In contrast, Hanea & Nane (2019) proposed a score with an exact distribution. Even though both these scores have attractive theoretical properties and proved comparable on real and simulated data, their behaviour is not fully understood. We will present new comparisons and evaluations of the two scores that will clarify the source of discrepancies. A better understanding of the separate scores is however necessary prior to engaging in these comparisons. Simulated and real data will be used to clarify the properties of these two calibration measures. This exercise will help define and refine what leads to a good vs. a poor calibration score and how important are the fine differences between the realisations of these scores (which are random variables). We also aim to identify those situations when neither score is either easy to interpret, nor reliable. **References** Cooke, R.M., 1991. Experts in Uncertainty: Opinion and Subjective Probability in Science. Environmental Ethics and Science Policy Series. Oxford University Press. A.M. Hanea, G.F. Nane, 2019, Calibrating experts' probabilistic assessments for improved probabilistic predictions, Safety Science, 118, 763-771, ISSN 0925-7535, <https://doi.org/10.1016/j.ssci.2019.05.048>.

4.08.T-03 Averaging Quantiles, Variance Shrinkage and Overconfidence

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Averaging quantiles as a way of combining experts' judgments is studied both mathematically and empirically. Quantile averaging is equivalent to harmonically weighting (HW) densities evaluated at quantile points (Bamber et al 2016, Colson and Cooke 2017). Although not attested in any guidance of which the author is aware, HW is often employed as a way of summarizing expert judgment data without introducing additional assumptions. It has been adopted by the COVID-19 ForecastHub (<https://covid19forecasthub.org/doc/ensemble/>) (Ray et al 2020, Cremer et al 2021). Examples of others using quantile averaging include (Christensen et al 2018, De Gooijer and Zerom (2019), De Vries and de Wal 2015, Flandoli et al 2011, Sayedi et al 2020, Kim et al 2021). It has been promoted as an alternative to equal weighting (Lichtendahl et al 2013). A variance shrinkage law is established between equal weighting (EW) and HW. Letting $AveVar$ be the mean variance of the empanelled experts based on elicited (or imputed) distributions, one can show that $Var(EW) \geq AveVar \geq Var(HW)$. Equality holds for the first inequality iff experts all have the same best guesses and holds for the second if and only if the experts have the same variance and their distributions are completely correlated. Variance shrinkage raises the question whether HW invites overconfidence. The TU Delft expert judgment data base [1] contains the 49 studies since 2006 involving 530 experts assessing, in addition to the variables of interest, 580 calibration variables from their field to which true values were known. Of these, 140 experts (26%) would not be rejected as statistical hypotheses at the traditional 5% level (Cooke et al 2020). The previous analysis has been extended to include HW combinations for each study. The following picture emerges: Whereas PW and EW as statistical hypotheses would be rejected at the 5% level on 3 resp 2 of the 49 studies, HW is rejected on 31 studies. On 19 studies rejection is at the 0.1% level. HW's informativeness on average is 50% greater than that of PW, which in turn is twice that of EW. [1] available from <http://rogermcooke.net/> This site also contains pre 2006 data with 45 studies involving 446 experts and 778 calibration variables. The earlier studies are of less uniform design.

4.08.T-04 The Assessment of Overall Uncertainty in Risk Assessments

Ullrika Sahlin¹ and Andy Hart², (1)*Lund University, Sweden*, (2)*Newcastle University, United Kingdom*

Uncertainty analysis is the process to identify and assess the combined impact of sources of uncertainty on uncertainty in the conclusion. This is called "overall uncertainty" and must be assessed by expert judgement because it should consider all sources of uncertainty, i.e. not only those that are addressed by the assessment model or statistical analysis. The assessment of overall uncertainty is a key requirement of the European Food Safety Authority's guidance on uncertainty analysis, because overall uncertainty is what matters most for decision-making. The outcomes of interest for risk management will always be uncertain to some degree. Qualitative expressions of uncertainty, such as likely or unlikely, are ambiguous and mean different things to different people. Therefore, EFSA's approach is to recommend that assessors *always try* to quantify the overall uncertainty using probability. This should always be possible, provided the outcome of interest is well defined, but can be challenging for those unfamiliar with the approach. This presentation shares experience and lessons learned from implementing overall uncertainty assessment, which may be helpful when applying it in other areas. There are well-established methods for eliciting expert judgements to quantify uncertainty using probability, but this will be new for many assessors and requires some training and familiarisation. Existing guidance refers mostly to eliciting probability distributions for parameters, but assessment conclusions are often framed as binary statements which require only a single probability. Experience suggests that experts find it easier to give a range of probabilities for overall uncertainty. Documenting the methods, evidence and reasoning for the assessment of overall uncertainty is essential for the transparency and credibility of the results. Care is needed to present and explain the results, specifying clearly the outcome of interest and its probability, and to avoid confusion with other uses of probability to express frequency, incidence or risk. For this reason, EFSA's Guidance on the communication of uncertainty has suggested expressing probabilities that quantify overall uncertainty for a conclusion as % certainty for the more probable outcome. Though developed in the area of food safety, the Chief Scientific Advisors to the European Commission have commended the approach to assess overall uncertainty as suitable for use in other fields.

4.08.T-05 Application of Data Analysis Based Expert Knowledge Elicitation in Deriving In Vitro Point of Departure of DNA Damage With Uncertainty From Three In Vitro Assays

Dawei Tang¹, Andrew White², Andrew Scott³ and Mona Delagrangé³, (1)*Unilever SEAC*, (2)*Unilever, United Kingdom*, (3)*Unilever/Safety and Environmental Assurance Centre SEAC, United Kingdom*

DNA damage can be caused by exposure to certain genotoxic chemicals. Traditionally, genotoxicity data has not been used for risk assessment, and recently, quantitative methods have been developed to estimate threshold concentrations which could potentially be used in genotoxic risk assessment. However, the methods do not adequately address the mechanism causing the adverse effect, nor do they characterise the uncertainty in the estimate. Based on the outcome of three previously reported *in vitro* assays relevant to DNA damage mechanisms following exposure of p53-positive HT1080 cell line to quercetin, this study aimed to derive a Point of Departure (PoD) for DNA damage - the Lowest Observed Effective Level to cause saturation of endogenous DNA repair capacity in HT1080 cells, using both data analysis and Expert Knowledge Elicitation (EKE). The assays were: 1) microarray (MA) assay, aiming to determine the concentration of quercetin above which, both global transcriptional changes and those related to DNA Damage Response (DDR) in the HT1080 cells were induced, 2) DNA Repair Centre (RC) assay, measuring fold change of DNA RCs in HT1080 cells at different concentrations of quercetin and 3) Micronuclei (MN) assay, measuring fold change of MN in the HT1080 cells at different concentrations of quercetin. Based on the assays' outcome, a Bayesian method was used to generate distributions of PoD for the DNA RC assay and MN assay, i.e., the concentration above which, DNA RCs and MNs were formed. For the MA assay, the concentration above which DNA damage-related gene transcription were induced can be revealed. Whilst the induction of DDR differential gene expression, the formation of DNA RCs and MNs are closely related to

DNA damage, none of them directly measure the PoD for DNA Damage. However, knowledge exists on the relationship between the assay outcomes and DNA damage. Therefore, the Sheffield framework based EKE was used to estimate PoD of DNA Damage from the assay outcomes and this existing knowledge. In summary, this work used data analysis and EKE to generate a distribution of PoD for DNA damage induced by exposing HT1080 cells to quercetin based on three *in vitro* assays' outcome. The principles outlined in the work can be applied in many areas, e.g., toxicological and environmental risk assessment, where expert knowledge can be elicited and analyzed to derive a probabilistic distribution of a quantity of interest when there is a lack of direct data or knowledge.

4.08 Expert Knowledge Elicitation in environmental assessments - subjective, but scientific (Poster)

4.08.P-Mo226 Aggregation of Bounded Probabilities From Judgements by Experts in a Food Safety Assessment

Ullrika Sahlin¹, Matthias Troffaes² and Andy Hart³, (1)Lund University, Sweden, (2)Durham university, UK, (3)Newcastle University, United Kingdom

In expert elicitation, experts are asked to make probabilistic judgements. Several organisations producing scientific advice are accepting judgements made with probabilities that are bounded (also called “imprecise” or “approximate”). Bounded probabilities can be easier to obtain when resources to perform elicitation are limited, or help to reach a consensus judgement among multiple experts (behavioural aggregation). Mathematical aggregation of bounded probability judgments from multiple experts poses, compared to aggregation of precise probabilities, an extra challenge for assessment. What approach to use depends on the purpose of aggregation, and to what extent it is desirable to allow the aggregated judgement to retain information about disagreement amongst members in the group. We review methods to aggregate bounded probabilities and demonstrate their applicability and differences, using an example based on a recent assessment in the area of food safety. In this example, multiple experts assess multiple endpoints that are then combined in an assessment to quantify uncertainty about the threshold dose for the most sensitive endpoint. Aggregation of bounded probabilities can be done by taking the convex hull, pooling of the bounds, aggregation that requires a higher order measure over probability, or updating of judgments per expert considering the judgments by all others (which requires a model for updating judgements). We show that these approaches for aggregation give different ranges between the bounds and that approaches requiring additional information usually result in smaller ranges. Current guidelines for expert knowledge elicitation lack instructions on how to aggregate bounded probabilities. Methods to aggregate bounded probabilities deserve more attention as they play a vital role in assessments using expert judgements where experts provide their judgements with bounds on probabilities.

4.08.P-Mo227 Encoding and Updating Uncertain Judgements Using Hybrid Elicitation and Quantile-Parametrized Distributions

Dmytro Perepolkin¹ and Ullrika Sahlin², (1)Lund University, Lund, Sweden, (2)Lund University, Sweden

Bayesian parametric inference is about updating the prior beliefs on parameters in light of the new data. *Elicitation of parameters* require that the expert understands the model and the role a particular parameter plays in it. An alternative is to elicit *predictions* about the “next observation”. Predictive elicitation makes no distinction between the randomness explained by the model (variability) and uncertainty about the parameters within the model. This makes it difficult to update the expert predictions with the data coming from the new observations. We propose a *hybrid elicitation* approach, where elicitation of the observable quantities is accompanied by the elicitation of the uncertainty around them. Hybrid elicitation, combining features of predictive and parametric elicitation, can be used to define the prior distribution for a model defined by a quantile-parametrized distribution. In this talk we present an example of the expert-elicited foods consumption distribution used for exposure assessment, and update it with the observations of actual consumption obtained from the food consumption database. Asking experts to provide their uncertainty about the elicited quantile-probability pairs is enough to quantify the uncertainty about the food consumption distribution, which can be particularly useful when food consumption data is sparse.

4.08.P-Mo228 Global Approaches to Identifying and Managing Apparent Emerging Chemical Issues

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The chemicals management regulatory systems have evolved over the last century, as different aspects have been modified in response to scientific developments. Chemicals are managed under international, national and state-level legislative instruments, usually on the basis of use (e.g. industrial chemicals, pharmaceuticals, pesticides and biocides, cosmetics), exposure (e.g. occupational health, contaminated land) or intrinsic properties (e.g. hazardous substances, persistent organic pollutants). Nevertheless, chemical issues may arise even within these developed regulatory systems that present potential risks to human or environmental health. These chemical issues may arise from previously unknown hazardous properties or environmental exposure profiles, or from unexpected changes to the exposure profile from new uses. Such issues may be identified through existing regulatory frameworks or can be triggered from scientific research or real-world events, reported in scientific journals or by mass media outlets. This poster summarises approaches taken by various regulatory jurisdictions across the world to identify and manage apparent emerging chemical issues. Information was gathered from discussions with regulatory scientists and reports from regulators and authoritative bodies. Several jurisdictions use quantitative approaches to identify emerging chemical issues, including biomonitoring, environmental monitoring and *in silico* modelling. Furthermore, a small number of jurisdictions have developed qualitative approaches in the recent years to supplement already robust chemical management programmes. These

range from surveys of expert panels to systematic monitoring of websites and published information. Many of the data-driven qualitative and quantitative approaches can be quite resource-intensive. Varying degrees of structure are seen in the use of expert panels to identify emerging chemical issues, ranging from ad-hoc discussions to systematic surveys utilising the Delphi method. Overall, there is no 'ideal' approach to identifying emerging chemical issues. Bespoke approaches to identifying emerging chemical issues are dependent on the available resources (i.e. time and budget), expertise and remit of the risk management organisation.

4.08.P-Mo229 Proxies for Uncertainty Quantification - Are Best Estimates Enough?

Tina Nane¹ and Roger Cooke², (1)DIAM, TU Delft, Delft, Netherlands, (2)resources for the future, United States

The Classical Model for structured expert judgment (SEJ) is an established method to elicit, validate and aggregate experts' uncertainty assessments. The Classical Model proposes performance-based mathematical aggregation of experts' distributions, using statistical accuracy and informativeness to objectively evaluate experts assessments. Along with individual expert performance, the model therefore enables the evaluation of the aggregated distributions, by using the same performance measures that were used to evaluate individual assessments. The Classical Model has been employed in numerous professional applications, and expert data is continuously documented by Roger Cooke[1], which enables a meta-analysis of the performance of various weighting schemes to aggregate experts uncertainty distributions or quantiles. A ample body of research demonstrated the superiority of performance-based aggregation in terms of overall performance, cross-validation analysis and forecasting performance. Nonetheless, many published expert judgment studies elicit only best guesses. Further, very large studies often employ SEJ only for the most important variables, other variables are assigned 'nominal values' or averaged best guesses from experts. Best guesses will remain part of the expert judgment repertoire. Here we address the question whether it is possible to leverage the above-mentioned validation research to obtain proxies for uncertainty quantification based only on best guesses. We propose several such proxies and investigate, using the collected expert data, the performance of those proxies with respect to statistical accuracy and a proxy measure for informativeness, and compare their performance to equal weighted aggregation of experts distributions. [1] <http://rogermcooke.net/>

4.09 Implementing Ecosystem Functions and Services Approaches into ERA to Improve Decision Making: What else is needed? (Virtual Only)

4.09 Implementing Ecosystem Functions and Services Approaches into ERA to Improve Decision Making: What else is needed? (Poster)

4.09.P-Tu202 Feasibility of Applying Ecosystem Services Principles to the Derivation of Environmental Quality Standards

Lorraine Maltby¹, Andrew Ross Brown² and Helen Wilkinson³, (1)The University of Sheffield, United Kingdom, (2)Exeter University, UK, (3)Environment Agency, England, United Kingdom

Environmental Quality Standards (EQSs) are key tools in the assessment and control of hazardous chemicals in the environment in order to protect biodiversity and ecosystems. To maximise their effectiveness, we need to ensure that EQSs are scientifically defensible and relevant. Currently EQSs used to protect freshwater ecosystems from the adverse impacts of chemicals are based on toxicity data generated from single species toxicity tests and are typically driven by the most sensitive species, irrespective of its functional role. The current approach takes no account of species interactions or ecosystem processes and hence the links between EQS, biodiversity and ecosystem functioning are uncertain. We describe the principles involved in adopting an ecosystem services approach to setting EQS values and then describe the technical steps involved. Two exemplar chemicals are used to illustrate the proposed approach. The regulatory implications for using ecosystem service-based EQSs in practice are outlined, their key benefits are highlighted and the technical requirements for their derivation and implementation are identified.

4.09.P-Tu203 Linking Individual Level Effects and Ecosystem Functioning Through Invertebrate Feeding: What Do We Know About Feeding Responses to Stressors in the Lab?

Niamh O'Connor¹, Philip Warren² and Lorraine Maltby¹, (1)The University of Sheffield, United Kingdom, (2)University of Sheffield, United Kingdom

For effective environmental protection, it is critical to understand how effects observed in the Laboratory toxicity tests translate into changes in ecosystem processes and functioning in the field. In freshwaters, invertebrates play a key role in the trophic transfer of energy, decomposition, and nutrient cycling. Impacts of stressors on invertebrates are likely to result in ecosystem level responses and changes in ecosystem service delivery. Changes in feeding rate is a general stress response and has been found to be a sensitive toxicity endpoint. It may also provide a link between toxicity effects at the individual and ecosystem level, using energy as currency. However, feeding rate is not a standard toxicity endpoint. A systematic review of studies examining freshwater invertebrate feeding rate responses to stressors was conducted to establish 1) the species in which this had been tested 2) the mechanisms by which stressors affect feeding rates 3) the variation in responses between species and contaminants 4) the sensitivity of feeding, compared to lethality, as an endpoint. A total of 395 laboratory feeding assays were found in 214 studies, observing the effects of 165 different contaminants across 7 Phyla (235 Arthropoda, 92 Mollusca, 29 Rotifera, 18 Cnidaria, 11 Platyhelminthes, 6 Annelida, 4 Nematoda). Most assays were conducted on only a few standard genera (Daphnia 97, Gammarus 56, Dressienia 20, Brachionus 18, Hydra 18). Fewer data were available for Insecta. Trichoptera and Odonata were the most studied but little, or no, information was available for other orders. About 80% of assays reported negative effects of contaminants on feeding rate and behaviour. No single mechanism was identified leading to reduced feeding but the mechanisms by which chemicals affect feeding are largely contaminant specific. Spearman's rank correlation was performed to compare the sensitivity

of the feeding endpoint to lethality ($r = 0.41$, $p < 0.01$). When grouping by mode of action, strong relationships were found for AChE inhibition ($r = 0.81$, $n = 80$) and polar narcosis ($r = 0.77$, $n = 20$) suggesting that species sensitivity of feeding response is positively related to sensitivity to mortality. This was not so strong for metals ($r = 0.24$, $n = 140$). These results suggest that, in the absence of feeding endpoints, lethality data may be used to predict feeding sensitivity for particular modes of action.

4.09 Implementing Ecosystem Functions and Services Approaches into ERA to Improve Decision Making: What else is needed? (Poster Corner)

4.09.PC-Tu13 Biodiversity Assessment Request Under Regulation EC No. 1107/2009 Approaches, Challenges and Limitations

Elena Alonso¹ and Miriam De Roman Martinez², (1)Knoell Iberia S.L., Madrid, Spain, (2)Dr.Knoell Consult GmbH, Germany
The legal framework that regulates the placing of Plant Protection Products (PPP) on the market (Regulation EC No. 1107/2009 and Regulations EC No. 283/2013 and EC No. 284/2013) includes the requirement to consider *the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects via alteration of the food web*. Besides, the *EU Green Deal, Farm to Fork and Biodiversity Strategies*, recently adopted by the European Commission, display objectives for reducing the loss of biodiversity and present quantitative targets by 2030 on pesticide uses, namely the reduction by 50% of the overall use of – and risk from – chemical PPP. However, at the time being, there is no methodology “fit for use” to satisfy the legal request of the biodiversity assessment within the PPP environmental risk assessment (ERA) scheme. Therefore in the present work, a review of currently available scientific reports and regulatory guidance has been conducted with the objective of identifying workable approaches to link available ecotoxicological data provided during the PPP authorization process with the assessment of biodiversity. According to this review, the Ecosystem Services (ES) concept is an existing available approach to identify Specific Protection Goals to support the biodiversity assessment, but it is still a conceptual idea without a clear methodology to be integrated into the PPP regulatory process. The ES concept, as well as the groups of organisms that provide these ES, are not clearly described in any guidance or publication and thus, limiting its direct link to standard Tier 1 toxicity data or even higher tier approaches. An additional challenging topic identified during this review is the lack of figures for discerning the effects on biodiversity caused by the use of PPPs from those caused by agricultural practices themselves, regardless of PPP application. This knowledge would be essential to propose appropriate mitigation measures that complement current ERA practices. Overall, this review provides a synthesis of the available conceptual approaches proposed by different stakeholders to address the impact of PPP on biodiversity. Based on that, it can be concluded that there are still many challenges and limitations before a workable regulatory ERA scheme for addressing biodiversity can be implemented.

4.09.PC-Tu14 PPP Precision-Application As Part of the Farm-To-Fork Strategy: Using Ecosystem Service Approaches to Assess Risks to In-Soil Organisms?

Steven Droge¹, Louise Wipfler² and Bas Buddendorff³, (1)Wageningen Environmental Research (Alterra), Netherlands, (2)Wageningen Environmental Research, Netherlands, (3)Wageningen UR, Nederland

The Farm to Fork (F2F) Strategy is a multi-level action plan as part of the EU Green Deal, addressing the challenges of sustainable food systems. Part of F2F is a targeted 50% reduction of the overall use and risk of chemical pesticides by 2030. Precision-farming can contribute to this target, supported by technical innovations, improving access to fast internet in rural areas and satellite technology. Precision-application of plant protection products (PPPs) can, besides reducing the amount applied, result in a lower risk of protection-targets. It will also introduce in-field spatial variability in exposure concentrations, particularly in a spot treatment. Taking these effects into consideration, precision-applications for crop protection could play a role in the environmental risk assessment (ERA) of PPPs. This presentation explores several key issues with implementing precision application as part of the ERA for in-soil organisms under the current regulations. Currently, the Tier 1 ERA for PPP regulation is based on negligible chronic effects on reproduction of three key species (earthworm, collembola, predatory mite), based on the local in-field maximum soil concentration (PECs). Yet, even for spot treatment applications (use on < 10% of the total field), PECs are based on the advised application dose considering the low dispersal capacity of in-soil meso- and microfauna. EFSA is currently revising Guidance documents on Persistence (9188/VI/97 rev.8) and Terrestrial Ecotoxicology (SANCO/10329/2002). Additionally, an EFSA Scientific Opinion on the science behind the soil RA was published in 2017. This Scientific Opinion addressed the implementation of specific protection goals for Ecosystem Services (ES) provided by agricultural soils, and provides arguments to better include external recovery (dispersal) and internal recovery (e.g. reproduction). Based on recovery rates, small effects up to a period of months may be tolerable for most service providing units. In case of precision-application, external recovery may occur from “unimpacted” patches to impacted field hot spots. The increased level of realism in ERA via an ES approach should also account for multiple stressors (e.g. including the full PPP treatment scheme per crop) and should be supported by adequate population modelling. Several examples of precision application are presented with options for recovery with considerations for spatial and temporal variability.

4.09.PC-Tu15 A Review of Environmental Protection Goals: What Are the Options for the UK?

Amy C. Brooks and Helena Crosland, Cambridge Environmental Assessments, United Kingdom

Protection goals are key to the environmental assessment of pesticides. Without them it is not clear what is being protected and whether it is appropriate to authorise or refuse a plant protection product. Protection goals must be clear regarding what is acceptable in terms of size/magnitude of effects in the environment of the substances being used and, furthermore, indicate what level of uncertainty is acceptable. General protection goals for the environmental risk assessment (ERA) of plant protection products are stated in relevant legislation but specific protection goals (SPGs) are not defined. However, as these are crucial for

carrying out a regulatory risk assessment and for making a decision, in certain circumstances they have been defined in guidance documents. The UK regulatory authority (HSE CRD) is funding a project to establish what “approaches” are currently available that will help with the definition of specific protection goals in the UK. This poster will present a summary of all the currently available approaches, including ecosystem services, for determining, and hence establishing, environmental protection goals for non-target organisms. An assessment of the main pros and cons of the approaches will also be presented. An opinion will be provided regarding the best methodology for establishing environmental protection goals for non-target organisms in the UK. The setting of specific protection goals will be part of a future project and won't be addressed here.

4.09.PC-Tu16 Functional Measures As Potential Indicators of Down-The-Drain Chemical Stress in Freshwater Ecological Risk Assessment

Claudia Rivetti¹, Laura Harrison², Katie Pearson³, Christopher Wheatley², Jane Hill², Lorraine Maltby⁴, Lucy Speirs⁵ and Piran White², (1)Unilever, Sharnbrook, United Kingdom, (2)University of York, United Kingdom, (3)Environment & Geography, University of York, York, United Kingdom, (4)The University of Sheffield, United Kingdom, (5)Unilever, United Kingdom

Conventional Ecological Risk Assessment (ERA) predominantly evaluates the impact of individual chemical stressors on a limited range of taxa, which are assumed to act as proxies predicting impacts on freshwater ecosystem function. However, it is recognised that this approach has limited ecological relevance. Here, we reviewed published literature to identify measures that could be considered as functional indicators of down-the-drain chemical stress as a potential approach to build more ecological relevance into ERA. Overall, we found wide variation in the use of the term ‘ecosystem function’, and conclude that it is important to distinguish between measures of processes (i.e. the interactions between biotic and abiotic components of the ecosystem) and measures of the capacity for processes (i.e. the status and composition of functional traits underlying processes). We present a classification of potential functional indicators and suggest that including indicators directly connected with processes will improve the detection of chemical stress impacts on ecosystem functioning. The rate of leaf litter breakdown, oxygen production, carbon dioxide consumption and biomass production show high potential to be used as functional indicators in ERA. However, the limited supporting evidence means that further studies are needed before these measures can be fully implemented and interpreted within ERA and with regulatory context. Sensitivity to chemical stress is likely to vary among functional indicators depending on the stressor and ecosystem dynamics context. Therefore, we recommend that ERA incorporates a variety of indicators relevant to each aspect of the function of interest, such as a direct measure of a process (e.g. rate of leaf litter breakdown) and a capacity for a process (e.g. functional composition of macroinvertebrates), alongside structural indicators (e.g. taxonomic diversity of macroinvertebrates). Overall, we believe that the consideration of functional indicators can add value to ERA by providing greater ecological relevance, particularly in relation to indirect effects, functional compensation, interactions of multiple stressors and the importance of ecosystem context.

4.09.PC-Tu17 Leaf Litter Decomposition As a Functional Indicator of Chemical Stress in Ecological Risk Assessment

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Using toxicity data from one or a few selected species to understand chemical impacts on whole ecosystems in ecological risk assessment is an approach based on the concept of protection from, rather than prediction of, chemical effects. As a result, it does not consider all potential impacts of chemicals on the complex processes and interspecies interactions present in ecosystems. Varying responses of different species to chemical exposure, and cascading ecological effects make outcomes difficult to predict at the community level. Measuring change in rates of ecosystem functioning provides an alternative approach and may make ecological risk assessment more representative of ecosystem-level responses. Leaf litter decomposition is a key ecosystem function which regulates carbon and cycles nutrients via feeding behaviour of detritivore species, which can be impacted by chemical exposure, making decomposition sensitive to the effects of chemicals. Using decomposition as a functional indicator of stress requires a good understanding of its underlying drivers, especially relationships between function and biodiversity. Utilizing an overview of published reviews, we examined whether a general consensus exists in the relationship between freshwater community biodiversity and leaf litter decomposition rate, by quantifying the reported presence and type (i.e. positive, negative, inconsistent) of the relationship. Our overview: 1) collated systematic reviews on the relationship between freshwater community biodiversity and decomposition, 2) assessed the methodological quality of reviews, and 3) determined the consensus on the decomposition-biodiversity relationships. We found a total of 7 systematic reviews containing 242 independent papers, with 5 out of 7 reviews reporting a positive relationship between biodiversity and the rate of decomposition, showing a consensus. The AMSTAR2 tool was used to assess methodological quality, revealing common gaps in review methods. For example, no reviews clearly reported a priori methods or reported that study selection and data extraction was performed in duplicate. Our findings will inform future work on evaluating the usefulness of leaf litter decomposition as an indicator of community responses to chemical exposure, by assessing whether rate of decomposition changes consistently with chemical exposure. This will inform development of decomposition as a robust functional indicator for use in environmental risk assessment.

4.09.PC-Tu18 Ecosystem Service Delivery in Freshwater Catchments: A Review of Current Tools and Methods and Their Potential Application in Risk Assessment

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Several challenges exist when applying an ecosystem services approach to ecological risk assessment. These include questions regarding the availability of methods and tools for applying an ecosystem services approach to chemical risk assessment and the

spatial and temporal scale of these approaches. Freshwater ecosystems and their catchments provide a wide range of ecosystem services (i.e. drinking water, fisheries, and recreation). However, they are exposed to both diffuse and point source pollution, which may adversely affect ecosystem service delivery. We conducted a literature review to identify approaches used in studies for ecosystem service quantification both within freshwater ecosystems and in the wider catchment. This review aimed to investigate: (1) Which freshwater ecosystem services are currently assessed? (2) What spatial and temporal scales are current tools capable of assessing? (3) Are there tools which can readily assess the effect of chemical stressors on ecosystem service delivery? The search returned 289 relevant studies with 117 unique mentions of models or tools on ecosystem service assessment. The approaches for ecosystem service assessment covered lentic and lotic freshwater ecosystems and catchments, as well as standalone wetland ecosystems. Results indicate that nearly all freshwater services were assessed with the majority of studies investigating regulatory & maintenance services (55%) such as water cycle and flow regulation. Concerning the spatial and temporal scale of studies, catchment-level assessments made up most (59%) of the studies whilst lentic and lotic systems were considered in about 20% of the studies. Multiple stressor types appeared within the studies including land use, climate change, and pollution. Pollution was assessed in about 16% of studies for different pollutants including nutrient loading, wastewater, and chemicals. Chemical stressors were not as widely acknowledged in literature as opposed to other stressors, making up a total of 9 studies from the search. In these studies, methods often employed several models and tools in order to assess chemical effects on ecosystem service delivery. This review has resulted in a database of ecosystem service tools and models for catchments as well as highlighting the need for model-based approaches to determine chemical stressor impact on ecosystem service delivery.

4.10 Linking soil ecology, ecotoxicology and risk assessment to the impact of multiple stressors on soil organism communities in the field (Part I)

4.10.T-01 ERAMYC - the Need of a Test System With Arbuscular Mycorrhizal Fungi Including Pre-Symbiotic and Symbiotic Phases for Environmental Risk Assessment

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Arbuscular Mycorrhizal Fungi (AMF) are recognized as ecologically relevant in supporting key soil functions, providing important ecosystem services (e.g. increasing resistance of host plant to several stressors, acquiring nutrients from soil for host plants, stabilizing soil structure and increasing soil carbon stocks). AMF have been indicated by the European Food Safety Authority as a potential group of non-target organisms for the risk assessment of Plant Protection Products. The existing ISO protocol 10832, which aims to evaluate effects of pollutants on AMF, considers the pre-symbiotic phase, only. Spore germination, germ tube formation, and asymbiotic hyphal growth are important phases of the fungal life cycle and are essential for establishing symbiosis. Some other processes like root colonization could be affected by pollutants and are not addressed by existing test protocols. Some studies have also analysed how stressors can influence AMF colonization, but at the moment, there is no standardized test protocol available which includes effects of soil contaminants on AMF species during the symbiotic phase. ERAMYC is a project which integrates a consortium of experts and aims to i) improve the existing protocol for the pre-symbiotic phase by including additional AMF species, test conditions and procedures embracing the symbiotic phase of AMF species; ii) standardize the developed advanced protocol through a ring-test with different chemicals; iii) develop a draft OECD Test Guideline and evaluate experimental results compared to the existing framework for the risk assessment of soil organisms exposed to chemicals. ERAMYC project is structured in four work packages (WPs): WP1 comprises a literature review; WP2 integrates two clusters of experiments to evaluate the performance/suitability of the selected AMF species in selected soils under specific test conditions and to assess how the different AMF species and parameters measured react to the selected chemicals; WP3 comprises a ring-test and WP4 aims at developing a draft OECD Test Guideline and a validation report for testing AMFs exposed to chemical substances. The literature review evidenced that sensitivity of test parameters can be species and substance dependent. The first phase of WP2 is ongoing and some preliminary results on performance/suitability of the selected AMF species in selected soils under specific test conditions (environmental conditions and selected host plants) will be presented.

4.10.T-02 How Is the Nitrogen Cycling Function of the Soil Affected by Copper Pollution in Two Contrasting Soils?

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Soil is a complex and dynamic ecosystem which holds a tremendous diversity of life forms that perform important processes. Nitrogen (N) is one of the most important macronutrients for plants that is cycled in soils via various microbial-driven processes. Site-specific studies have shown that stressors such as pollutants may affect the abundance of specific functional groups of N-cycling organisms. However, it is unknown if these effects are generalizable among soils with different properties. Moreover, the microbial abundance only reflects potential functioning, but actual activity under field conditions and its link to plant productivity is hard to reveal. The present study addresses this complexity by assessing effects of copper (Cu), a common heavy metal pollutant, on the N cycle in two different soils with highly contrasting properties. Microbial parameters are studied in concert with plant parameters so that changes in communities of N transforming microorganisms may be linked to plant quality and quantity. A

lysimeter experiment was performed in 2015-2017 as part of the APPLICERA project. Two pasture soils, one acidic sandy soil and one slightly alkaline sandy loam soil were spiked with a CuCl₂ solution and aged six months before mixing with untreated soil of the same origin until target concentrations (resp. 0, 200, 430 and 1100 mg/kg Cu). The soils were placed in lysimeters and sown with a grass mixture of *Festuca rubra* and *Poa pratensis*. Plants and soils were sampled in the second growing season (2017). Abundances of the microbial N-cycling functional guilds ammonia-oxidizing archaea and bacteria (AOA and AOB), nitrite-oxidizing bacteria *Nitrobacter* (NIB) and *Nitrospira* (NIS), and denitrifiers were determined by real-time quantitative PCR (qPCR), and then compared to measurements of plant shoot- and root biomass, C (carbon), N and stable isotopes ¹³C, ¹⁵N, and ¹⁸O. Negative effects on the microbial communities and plant biomass were much more pronounced in the acidic sandy soil. Remarkably, the effects on plant N, C/N and ¹⁵N were similar between soils, while the abundance of microbial communities had very distinct soil-specific patterns. This may speak against generalizing microbial community responses to pollution stressors across soil types. Also, plant parameters did not correlate well with microbial abundances, which shows that other pathways and communities may also play large roles in the soil N cycle under stressed conditions.

4.10.T-03 Investigating the Effects of Pesticides on Soil Microbial Communities Using rRNA Amplicon Sequencing

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It should be ensured that the application of pesticides to agricultural soils does not harm these communities. Potential effects comprise structural as well as functional change. However, soil microbial communities naturally have a high variation of these traits. This complex and chaotic network is far from being fully understood. Luckily, next-generation amplicon sequencing allows scientists to drastically improve their insight into the genetic structure of soil microbial communities in the last decade. Can this technology help us regarding the environmental risk assessment of pesticides? Analyses of the soil bacterial community of soils will be presented showing that the herbicide MCPA and fungicide Metalaxyl-M did not have clear and dose-related effects. Also, data on the response of fungi will be presented (data are currently processed). Structural and functional investigations both are required for evaluating the effect of pesticides on communities. Additionally, the talk will cover the possibilities, limitations, and biases during the different stages of laboratory and computational work. Finally, the suitability and value of amplicon sequencing data for evaluating the environmental risk of pesticides for soil microbial communities in the regulatory context is discussed.

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4.10.T-04 Determination of Soil Invertebrate Diversity Using Morphological and Dna-Based Methods at 25 Sites in Germany

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The recording and evaluation of the diversity of soil organism communities have made considerable progress within the last decade (see, e.g., the results of the European FP7-project „EcoFINDERS“). In particular, the large influence of these organisms on the ecological functions of the soil has been highlighted (e.g., maintenance of soil structure and organic matter decomposition). Equally important, it was shown that not only flagship species such as the deep-burrowing earthworm *Lumbricus terrestris*, but also the highly diverse meso- and microfauna have to be considered. However, in Germany no regional coverage and comprehensive information regarding the biodiversity of soil invertebrates is available. Reasons for ignoring them are the lack of trained taxonomists, lack of sampling methodology standards (released only recently by ISO) as well as the general lack of interest in these often small and inconspicuous species. In order to improve this situation, the German Federal Environmental Agency (UBA) started a project in 2018, aiming to develop a standardized, comprehensive and efficient programme to monitor selected soil invertebrate groups all over Germany, representing different soils, land use forms (i.e., meadows, crop sites and forests) and regions. Sampling was performed at 25 Permanent Soil Monitoring Sites (PSMS), looking in particular at earthworms (Lumbricidae), springtails (Collembola) and potworms (Enchytraeidae), using ISO standard sampling methodology. Species belonging to these groups were determined by classical morphological methods but also by using modern genetical methods such as DNA-metabarcoding of a mixture of soil invertebrate communities (comDNA) as well as metabarcoding of environmental DNA (eDNA) extracted from soil. The final aim of the project is to develop an efficient, cost-effective, and routinely applicable soil invertebrate monitoring system, as a tool for the evaluation of soil invertebrate biodiversity under stress. A pilot study was conducted in autumn of 2018 at one meadow, one crop site and one forest in order to test the experimental approach and decide on the sampling design for the remaining 22 sites. The project progress was severely slowed due to the Covid pandemic. The sampling programme was completed in autumn of 2021 and the final samples are currently being processed. Selected results from the project will be presented.

4.10.T-05 Chemical Sensitivity of Earthworms: Effects of Morphology, Phylogeny and Habitat

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The chemical risk assessment (RA) of plant protection products on non-target soil macro-organisms has mainly been done by testing the acute, chronic and sublethal responses on the compost earthworm *Eisenia fetida*. Although *E. fetida* meets the basic

requirements for being a RA standard test organism, its use as the only earthworm representative has often been criticized as being only to a limited extent representative for other species found in the field. In this context, it has been proposed to include additional species covering the three main ecological groups of earthworms in the RA process (i.e. endogeic, anecic and epigeic). As toxicity data for other earthworm species is scarce, the aim of this research was to provide information on the chemical sensitivity of multiple earthworm species. Hence, acute single-species tests of earthworms sampled from different habitats (i.e. forest, wetland and grassland) and covering the major ecological groups were performed based on the artificial soil test described in the OECD 207. The median lethal concentrations (LC₅₀) of a copper-based fungicide and the insecticide imidacloprid were derived. Additionally, species sensitivity distributions were fitted together with the hazardous concentration affecting 5% of the species (HC₅). In both pesticide assessments, laboratory-raised *E. fetida* mortality responses were less sensitive than most of other earthworm species, including *E. fetida* collected in the field. Additionally, the HC₅s calculated for copper and imidacloprid were 173.36 and 0.58 mg a.i. / kg d.w., respectively. Preliminary results suggest that the chemical sensitivity of earthworms is linked with environmental variables such as soil pH, where earthworms sampled from acidic soils were more resistant to both substances. Experiments covering a wider range of species is required to confirm and understand the relationship between habitat variables and chemical sensitivity.

4.10 Linking soil ecology, ecotoxicology and risk assessment to the impact of multiple stressors on soil organism communities in the field (Part II)

4.10.T-06 Carbon Dioxide Effects on the Soil Invertebrate *Enchytraeus crypticus* Exposed to Metal(Loid) Contamination

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Greenhouse gas emissions (e.g., CO₂) by human activities are causing serious environmental impacts worldwide. Atmospheric CO₂ levels are predicted to reach 800-1000 ppm by 2100, which might exacerbate global warming. The impact of global climate changes might be aggravated in environments affected by metal(loid) contamination where soil living organisms have to deal with multiple stressors. Thus, the aim of the present study was to evaluate the effects of increased levels of atmospheric CO₂ on the soil invertebrate *Enchytraeus crypticus* exposed to a metal(loid)-contaminated soil, from sub-organism to population level. *E. crypticus* was exposed for 48h (short-term exposure) and 21d (long-term exposure) to a metal(loid)-contaminated soil from central-northern Portugal under different CO₂ levels based on the IPCC predictions for 2100. The scenarios tested were: standard climate conditions as recommended by OECD/ISO guidelines, 20°C+50% soil water holding capacity (WHC)+400-500 ppm CO₂; low exposure CO₂ level: 20°C+50% WHC+600 ppm CO₂; intermediate exposure level: 20°C+50% WHC+800 ppm CO₂; high exposure level: 20°C+50% WHC+1000 ppm CO₂. After the short-term exposure, surviving *E. crypticus* were analysed for body As and Pb concentrations, RNA content, expression of target genes related to stress conditions (metallothionein-1 -MT1-, catalase-1 -CAT1-, and heat shock protein-70 -Hsp70-), and biochemical markers related to stress conditions (activity of the enzymes cholinesterase -ChE-, catalase -CAT- and glutathione-S-transferase -GST- and of the electron transport system -ETS-, and lipid peroxidation levels -LPO-). After the long-term exposure, *E. crypticus* survival and reproduction were assessed. Soil pH, electrical conductivity, and water-extractable metal(loid) concentrations were also analysed. Short-term exposure to metal(loid) soil contamination and increased levels of atmospheric CO₂ favoured the bioaccumulation of metal(loid)s in *E. crypticus*, which was accompanied by significant changes in the expression of some target genes related to stress conditions (mostly MT1 and CAT1), but less changes in biochemical markers were observed. Despite this, when exposed to this multi-stressed scenario for a longer period, few effects at population level (survival and reproduction) were noticed. This study emphasises the relevance of considering future climate changes under real contamination scenarios.

4.10.T-07 Soil Moisture Interferes With Uptake and Effects of Phenanthrene in Springtails

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Drought is one of the most important environmental factors influencing the physiology of soil invertebrates. Springtails (*Collembola*) are a species rich group of invertebrates inhabiting soil, litter or vegetation of most terrestrial ecosystems. The springtail, *Folsomia candida*, is often used in standardized ecotoxicity testing. The drought physiology of *F. candida* is well known and therefore *F. candida* ideally suitable for both drought stress and soil toxicity tests. Here, we employed a long-term exposure to phenanthrene (PHE) and a series of soil water contents to study the combined effects of these stressors/factors to survival, reproduction and biomass in a full factorial experiment. Our results showed that the used concentrations of PHE had no significant effect on adult survival rate ($p > 0.05$) whereas very dry soil (soil water content at 4% of dry weight) caused a significant decrease of survival rate. Growth of springtails was significantly lower at PHE 60 mg/kg dry soil than in uncontaminated soil ($p < 0.05$), and growth was very low when soil water content was at or below 6% of dry weight. Reproduction of springtails was susceptible to drought and PHE and their combinations in this study. Juvenile counts significantly decreased with soil water content decreasing ($p < 2e-16$) and PHE increasing ($p < 2e-16$). Moreover, we observed a synergistic interaction of these two stressors significantly impacting juvenile counts ($p < 2e-16$). Bioaccumulation factor (BAF) decreased significantly with soil water content increasing at PHE 10 mg/kg dry weight, 20, 30, 40 and 60, corresponding to p values 0.0004128, 1.4e-05, 8.26e-10, 0.00632 and 5.35e-06, respectively. Soil water content has significantly impacted BAF that increased almost 3-fold from 22% to 5% soil water content. Taking the final concentration of PHE in soil into account, we found that bioaccumulation (BAF) decreased significantly with moisture increasing at PHE 30 mg/kg dw. The results show synergistic

interaction of soil water content and PHE for reproduction after 28 days. Moisture levels can interfere with the PHE concentrations in animals, indicating that drought could weaken PHE detoxification, but strengthen PHE accumulation in animals.

4.10.T-08 Intermediate Tier-Based Approach in the Environmental Risk Assessment by Using a Set of Natural Reference Soils in OECD 222 Earthworm Reproduction Test

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One of the most used laboratory tests at lower tier for soil organisms in the environmental risk assessment is the earthworm reproduction test OECD 222 with *Eisenia fetida/andrei* in artificial soil. By using a unique artificial soil, test system criteria such as reproducibility and comparability are met. However, one artificial substrate cannot represent the diversity of natural soils, nor is it representative of arable soils to which plant protection products are applied. Moreover, the sorption properties of a soil can influence the bioavailability of chemical substances and consequently their toxicity for soil organisms. If soil parameters are not included in the examinations, it can lead to a bias in the results. If a potential risk for the soil organisms is expected from extrapolations of the laboratory results to field conditions, higher-tier studies are carried out. In these earthworm field studies performed according to ISO 11268-3 (1999), it is recommended to avoid the use of extremely sandy or clayey soils. Further criteria for the soil parameters are not set. This lack of consideration of soil parameters represents a gap in the environmental risk assessment of chemicals that is currently neither covered by laboratory studies conducted with artificial OECD substrate, nor by field studies usually performed only on one of many possible arable soils on one site. To fill this gap, a set of natural agricultural reference soils (RefeSols, Fraunhofer) with a broad spectrum of soil properties that can be found in agricultural soils in mid-latitudes were evaluated in the lab with the reference substance carbendazim in the OECD 222 test system and compared to the OECD substrate. A successful, valid and reproducible implementation of a set of natural agricultural reference soils in the OECD 222 test system was possible. The use of field soils in extended-laboratory tests with various properties provides a more realistic application-oriented approach, and can help in refining the risk assessment and eventually avoid field trials. The study results provide to estimate a spectrum of possible risks that may occur in diverse agricultural soils rather than a single toxic endpoint on OECD soil with uncertainties.

4.10.T-09 Multiple Pesticide Residues Across the European Terrestrial Ecosystems: Risks and Challenges

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Farming systems in Europe rely strongly on the use of Plant Protection Products (PPPs) to secure yields and food safety in plant production and animal husbandry, using an average 340,000-370,000 tons of active substances per year. Multiple PPP residues are then commonly found in soil, water, crops, food and feed, animals and humans. Of the 487 substances currently approved in the EU market and combined in several thousand different commercial PPPs, almost 50% are bioaccumulative and 25% are persistent in soil (PPDB, 2019). According to the Classification, Labelling and Packaging Regulation (CLP), 30% have a high acute aquatic toxicity and 28 are suspected carcinogens. These properties, among others, are potentially harmful to ecosystem, plant, animal and human health. However, data on the distribution of PPPs across European agricultural landscapes, that account for ecological and environmental variability, are scarce and fragmented (Silva et al. 2019). There is a need to harmonise data collection approaches across Europe and collect the critical data necessary to deliver integrated approaches to fully assess overall risks and impacts of PPP formulations, residues and their metabolites. The overall aim of SPRINT project (SPRINT-h2020.eu) is to develop, test, validate and deliver a Global Health Risk Assessment Toolbox for the integrated assessment of the impacts of PPPs [formulations (F), active substances (a.S.), metabolites (Me) and mixtures (Mi)] on terrestrial and aquatic ecosystems (E), plant (P), animal (A) and human (H) health. Three main attributes for the health status will be examined: resilience, reproduction/productivity and manifestation of diseases, while transition pathways towards sustainable use of PPPs will be identified in a multi-actor approach. In this study we present first results on (multiple) pesticide distribution in the terrestrial environment based on a harmonised data collection conducted in the growing season 2021 in 10 representative Case Study Sites (CSS) across Europe covering the main EU cropping systems. We correlate the measured pesticide concentrations in the soil with recorded application data per crop and farming system. These field data can be used as a verification for the EFSA predictions (predicted environmental concentrations in soil), and as a basis for new terrestrial ecotoxicological tests.

4.10.T-10 Influence of Plant Protection Products on Beneficial Soil Life

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Plant protection products (PPPs) are indispensable in today's agriculture. However, the contamination of soils with PPP raises concerns about effects on beneficial soil life. So far, few reports are available on PPP residues in agricultural soils with regular pesticide applications and almost no data can be found on residues in organically managed soils or grassland where they are not or no longer applied. However, knowledge about these areas is of utmost interest, as these as these soils are considered particularly vital and ecologically valuable and may serve as ecological sanctuary for soil organisms. We assessed the occurrence of 45 PPPs or metabolites in 120 agricultural soils. We specifically tested whether the occurrence of pesticides differed between organic and conventional management and whether it is dependent on the landuse type (vegetable farming, arable farming and grassland). Furthermore, it was investigated whether the occurrence of residues is linked to important indicators of soil life. In a follow up greenhouse study, the effect of the bioavailable fraction of three fungicides on soil microorganisms was assessed. Residues were

found in all sites, pointing towards a consistent background contamination of soils no matter the management practice. Also after 20 years of organic farming without application of synthetic PPPs, up to 16 different residues were found. Various biological endpoints, including the abundance of beneficial arbuscular mycorrhizal fungi, were significantly negatively linked to the amount of residues in soil, indicating that PPPs are a key factor determining microbial soil life. The greenhouse setup showed that the bioavailability of the three different fungicides was similar between the tested soils, independent of their soil properties. Furthermore, some of the examined microbial genes reacted to the bioavailable fraction of the fungicides, thus being sensitive enough for changes to be evident within the observed time period. The results of the field study demonstrated that PPP are a hidden reality in agricultural soils and suggest that they have harmful effects on beneficial soil life. This widespread contamination of PPP in all soils raises the question of how bioavailable these PPPs are in these sites and whether they still influence important soil organisms and functions. Future analysis from the greenhouse study, including the assessment of functional gene abundance will clarify this.

4.10 Linking soil ecology, ecotoxicology and risk assessment to the impact of multiple stressors on soil organism communities in the field (Virtual Only)

4.1.V-01 A Field Study on Soil Ecological Risk Assessment Based on TRIAD Approach

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In 2017, International Standard Organization (ISO) published the guideline for site-specific ecological risk assessment (SERA) based on TRIAD approach. The TRIAD approach is required three lines of evidence (LoE); chemical-LoE (Chem-LoE), ecotoxicological-LoE (Ecotox-LoE), and ecological-LoE (Eco-LoE) and integrate them as one risk number; integrated ecological risk (IR) on scales of 0-1. This study performed site-specific SERA of metal contaminated area based on TRIAD approach. This study attempted to minimize uncertainty in the estimation of IR by constructing far more assays than previous studies. The aim of this study is to suggest priority of assays representing ecological risk. For Chem-LoE, total concentrations and extractable concentrations of heavy metal was measured. Six soil bioassays (including two plants, earthworm, collembola, soil nematode, and soil algae) and five soil extract bioassays (including fish embryo, waterflea, nematode, algae, and microorganism) were conducted for Ecotox-LoE. In Eco-LoE, bait-lamina test, collembola abundance, vegetation survey was performed. The IR was estimated by assigning the equal weight to the three-LoEs. As a result, it was confirmed that despite the high chemical risk, it may appear differently from the actual ecological risk through mutual supplementation with Ecotox- and Eco-LoE. The results of this study emphasized the validity of TRIAD approach for site-specific SERA by confirming the harmony of three LoEs. This study provides useful information on future policies for SERA assessment and risk management in that it suggested the priority of tools in SERA by presenting assay that can represent ecological risk. *Acknowledgement-This study was funded by the National Institute of Environment Research (NIER) of the Republic of Korea (NIER-SP2020-223).*

4.1.V-02 A Multiparametric Comparison of the Toxicity of Non-Encapsulated and Microencapsulated Forms of Esfenvalerate in *Caenorhabditis elegans*

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The agrochemical industry has aimed to develop environmentally and user-friendly pesticide products, namely, new types of formulations such as the suspensions of microcapsules. The encapsulation of the active ingredients (AIs) has been described as a feasible approach to improve the targeting and delivery of these substances to the target pests/weeds, thus decreasing the quantity required for their intended pesticidal activity. However, there is a gap in the literature regarding the comparative health and environmental effects of encapsulated and non-encapsulated pesticides. In this context, the laboratorial model organism *Caenorhabditis elegans* was used to comparatively assess the effects of non-encapsulated and microencapsulated esfenvalerate (analytical standard vs. commercial formulation *Onslaught*) regarding different parameters. Specifically, the post-exposure reproductive capacity (brood size) and lethality were measured after 1-h exposure in aqueous medium to recommended application rates of this insecticide (0.025 % and 0.050 % AI), with subsequent change to OP50 *Escherichia coli*-seeded nematode growth medium plates for a recovery period. Additionally, the chemotaxis to *E. coli* – i.e. clean and contaminated by esfenvalerate in both forms at these same application rates – was comparatively assessed at 1 h, 3 h and 24 h of exposure. The effects of a microencapsulated pesticide on chemotaxis behavior in the nematode *C. elegans* were addressed for the first-time. We found that *C. elegans* was highly tolerant to both non-encapsulated and microencapsulated esfenvalerate with respect to lethality and reproduction, but its chemosensation response to bacterial food was similarly negatively affected by exposure to esfenvalerate in both forms. These results suggest that encapsulated formulations may not be environmentally safer options as argued by agrochemical industry. Given that chemotaxis behavior plays an important role in the survival of nematodes and other soil organisms (e.g. linked to the search for food and avoidance of toxic substances), a more profound understanding on the comparative effects of microencapsulated and non-encapsulated formulations to non-target organisms is of key relevance and should be addressed in future studies.

4.1.V-03 Assessment of Adverse Effects of Olive Mill Waste WATER and Olive Mill Waste Contaminated Soil on Springtail *Folsomia candida*

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The olive oil industry generates considerable amounts of olive mill wastewater (OMW) which is treated and used in agriculture, energy production, or discharged into evaporating ponds where OMW contaminated soil (OMWS) is formed. Due to the extremely high phenol content, untreated OMWS is not suitable for plants and soil organisms. Collembolans are an extremely important group of non-target soil organisms that are frequently used in ecotoxicological research. However, information on the impact of OMW on the collembolan species *F. candida* is scarce. Therefore, this study aimed to determine the effects of OMW and OMWS on survival, reproduction, neurotoxicity, oxidative stress, and available energy in springtail *F. candida*. Exposure to different ratios of OMW and OMWS showed a higher toxicity of OMWS in terms of survival ($LC_{50}=32.34$ % of OMW; $LC_{50}=45.36$ % of OMWS) and reproduction ($EC_{50}=10.10$ % of OMW; $EC_{50}=19.44$ % of OMWS). The number of juveniles gradually decreased as the ratio of OMW increased. In contrast, exposure to OMWS enhanced the reproduction rates in a lower ratio. However, complete inhibition of reproduction was observed after exposure to the highest ratio of OMWS (50%). Furthermore, neurotoxicity (AChE induction), oxidative stress (SOD, GST and MDA induction), and changes in available energy (decrease in lipid and carbohydrate content) have been observed. These negative effects are probably consequences of the high phenol content specific for OMW and OMWS at higher tested ratios. Namely, at lower ratios, OMW and OMWS served as a food source and had a positive effect on reproduction. Consumption of energy for reproduction, defense against neurotoxicity and oxidative stress has led to metabolism imbalance and consequently to long-term consequences visible at the population level. Obtained results indicate that for the ecotoxicological assessment of different wastes, the incorporation of ecotoxicological tests with non-target soil organisms is crucial. Moreover, it is essential to use different endpoints to gain insight into the exact mechanism of action. Furthermore, the application of OMW and OMWS in small ratios can have a positive effect on non-target soil organisms and consequently cause better quality of the soil. Therefore, more research is needed to monitor the impact of lower concentrations of OMW and OMWS over several generations to allow its safe application.

4.1.V-04 Biomarker Responses of *Enchytraeus Albidus* to Fungicide Azoxystrobin: Differences Between Active Ingredient and Commercial Product

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Strobilurin fungicides (SFs) are a group of new fungicides produced by strobilurin A as a lead compound. Azoxystrobin, the best-selling SF, is a highly efficient broad-spectrum fungicide with relatively high-water solubility, which can lead to accumulation in water, sediments, and soil. Due to its wide application and impact on non-target soil organisms, azoxystrobin has attracted attention. Soil dwelling organisms are among the first in line of exposure to pesticides. However, their risks are often based on the pure active ingredient (AI) and not on the commercial products (CPs) actually applied in the field. *Enchytraeus albidus* is a model species in soil ecotoxicology used for the assessment of environmental risk. Due to their important role in proper soil functioning, it is essential to investigate the toxicity and adverse effects of fungicides used in agriculture on enchytraeids. Previous research has demonstrated a negative impact of azoxystrobin on the reproduction and oxidative status of earthworms, and the survival, reproduction, and hatching success in enchytraeids. However, most studies have assessed the effect of AI, while only a few use a CP. Therefore, we investigated the effects of pure AI, azoxystrobin, and CP Quadris on survival, reproduction, MXR activity and oxidative stress biomarkers in *E. albidus*. The results showed that CP exhibited higher toxicity in terms of survival ($LC_{50}CP = 16.7$ mg/kg soil, $LC_{50}AI = 15.3$ mg/kg soil), while reproduction was affected only by CP ($EC_{50} = 3$ mg/kg soil). Furthermore, while AI caused induction of the multixenobiotic resistance system, CP caused inhibition. Moreover, although both tested substances caused oxidative stress, the toxicity of the CP was higher and long term impact in form of lipid peroxidation was observed. These results suggest the importance of evaluating the effect of AI and CP. Furthermore, to obtain more accurate information on the mode of action, access is required through different endpoints. These findings highlight the need to further explore the toxicity of CPs compared to AIs, in order to predict a more realistic environmental hazard of fungicides.

4.1.V-05 Field-Realistic Doses of the Neonicotinoid Acetamiprid Impact Natural Soil Arthropod Community Diversity and Structure

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The neonicotinoid acetamiprid is used as a foliar spray, this results in direct exposure of the soil and the organisms that maintain the soil health. It is already shown in lab tests that acetamiprid is toxic to the Collembola (springtails) species *Folsomia candida*, but Acari (mites), on the other hand, seem to be insensitive to neonicotinoids. This study determined: (i) if field-realistic doses acetamiprid affect the abundance and diversity of soil arthropod communities, and (ii) whether or not potential effects are short-term or persist after degradation of acetamiprid. Intact soil cores were taken from an untreated grassland field in Wageningen, the Netherlands, and placed in the CLIMECS (a standardized and controlled mesocosm set up). Thereafter, the naturally sourced communities were exposed to a control and different field-realistic doses (0, 0.05, 0.2, 0.8 mg a.s./kg dry soil) of acetamiprid were applied. After 10 and 56 days the abundance of mites and springtails was assessed. Simultaneously, for the springtails, diversity was assessed by identifying them to the lowest possible level. This data was, among others, used to analyse the effect of acetamiprid application on abundance over time, but also to analyse the changes in Alfa and Beta-diversity (PERMANOVA) as well as community structure. Springtails and mites abundances were more or less equal at the start of the experiment. After 10 days increasing doses of acetamiprid significantly lowered springtail abundance and increased mite abundance. At the highest dose, springtails decreased by 53% on average while mites increased by 26%. This effect was no longer clearly visible after 56 days, this might indicate a recovery of the springtail community. The relative proportion of mites in the total soil fauna actually significantly increased because of these strong declines in springtails, due to a lowering of interspecific competition (i.a. food or

living space). The community composition was significantly changed by acetamiprid and time. Shortly after application, communities diverged, from the control in terms of structure (Beta dispersion), while later, communities converged again, probably due to recovery of species that were not filtered out by the treatment and by the hatching of eggs. With this experiment, we are the first to show that field-realistic applications of neonicotinoids negatively impact natural soil fauna, both in terms of abundances as well as diversity and community structure.

4.1.V-06 Increased Temperature Enhances Toxicity From Contaminant Stress in a Widespread Collembola With Different Thermal Adaptation

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Springtails (Collembola) are important components of soil ecosystems worldwide, and may be exposed long-term to anthropogenic contaminants. As global air temperature continues to increase, it is likely that it will affect ectotherm responses to contaminant stress. Populations inhabiting different climates may respond differently to higher temperature, as their adaptation to utilize ambient heat likely differ. Our aim was to document whether sub-lethal effects on Collembola exposed to an insecticide depend on the ambient temperature. Additionally, we aimed to determine effect from the thermal adaptation of the study organisms on their response. One temperate and one arctic originated population of *Hypogastrura viatica* (Collembola) were exposed dietary to two sub-lethal concentrations of imidacloprid in addition to control, at 10, 15, 20 or 25°C. The exposure lasted from 0 to 1500 day-degrees (days*temperature), allowing comparisons between temperatures. Each experimental unit contained 25 juveniles, and each treatment had 12 replicates, of which 4 were harvested at three different life stages. We studied among other the traits survival, growth (body size) and egg production. Dietary exposure to imidacloprid resulted in sub-lethal effects for both populations at 10 and 15°C (>85% survival), while the highest concentration of 0.16 mg/kg dry bark was lethal at 20°C and 25°C (approx. 50-70% survival). The effect was stronger for the temperate population. The growth pattern for both populations showed little or no effect on the body size at an early age, but a strong reduction in body size at a high adult age, which was increasing with increasing temperature. A decrease in egg production in arctic *H. viatica* was caused by imidacloprid exposure of the highest concentration, but without any apparent interaction from ambient temperature. Contrastingly, the toxic effect on egg production was temperature-dependent for temperate *H. viatica*, for which higher temperature resulted in a stronger decrease in number of eggs laid. The temperate population is adapted to efficiently utilizing a warm summer climate by increasing egg production at increasing temperature, unlike the arctic population. The energy cost of high egg production might cause the temperate population to be more sensitive to other stressors when the temperature is high. Knowledge on how other factors affect toxicity from contaminant stress can contribute to a more relevant risk assessment in the future.

4.1.V-07 Linking Scavengers and Decomposers in the Anthropocene: Vertebrate Guilds Drive Soil Ecosystem Processes

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Currently, rewilding in Mediterranean ecosystems is driving the expansion of both native and exotic ungulates which are an important source of carcasses for scavenger vertebrate guilds. Carrion is an ephemeral resource of great quality that drives relevant ecosystem processes. Carrion consumption rates are influenced by scavenger communities, particularly species richness and the presence of key species such as vultures and top-predators. In this way, the heterogeneity derived from vertebrate communities, environmental factors and the type of carrion resource can significantly influence the interactions between scavengers, decomposers and soil properties. These interactions have not been addressed to date despite their potential regulatory role of key ecosystem processes. We evaluated some physicochemical and biochemical soil properties and the abundance of soil microbial communities before and after the consumption process of carcasses of Barbary sheep (*Ammotragus lervia*), an exotic invasive ungulate in the Regional Park of Sierra Espuña (SE Spain). Our results suggest that there are important differences in the soil properties studied and the activity of decomposing microorganisms related to the consumption rates and the richness of scavenging vertebrates in Mediterranean ecosystems. Particularly, carcasses influenced available phosphorus in the soil, basal soil respiration and microbial soil communities, especially the group of bacteria. The use of ungulate carcasses by vultures modulated the effects on soil ecosystem processes by enhancing carcass consumption rates.

4.1.V-08 Nature-Based Approaches for Oil-Impacted Ecosystem Restoration Using Microbes

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Petroleum hydrocarbon degradation potentials of bacteria isolated from crude oil-polluted site in Igbuduya IV (Lat N 5° 2' 57.2964" Long E 6°35' 5.7192) in Rivers State, Nigeria were investigated. Polluted soil samples were collected from four coordinate points using soil auger at depths of 0-15cm (surface) and 15-30cm (subsurface) soil while unpolluted control was taken 80m away from the polluted site. Physicochemical parameters of the soil sample were determined using APHA standard methods, while culture-based enrichment methods were used in bacterial isolation. Hydrocarbon degradation potentials of the bacterial isolates were determined by screening in Bushnell Haas broth amended with 1% crude oil and monitored weekly over 28 days. Optical density readings were taken on days 0, 7, 14, 21 and 28 using a calibrated spectrophotometer (at 580nm wavelength) to measure microbial growth and pollutant degradation. Three isolates with high degradation abilities were identified as *Lysinibacillus fusiformis*, *Bacillus thuringiensis* and *Micrococcus luteus* by amplification and sequencing of the 16S rRNA genes. The percentage degradation of crude oil by *Lysinibacillus fusiformis*, *Bacillus thuringiensis* and *Micrococcus luteus* were 97.93%, 97.44% and 97.69% respectively. Paraffin oil degradation was highest for *Lysinibacillus fusiformis* 97.56% followed by *Bacillus thuringiensis* 95.19% and *Micrococcus luteus* 94.85%. In conclusion, members of the indigenous microbial community in the

polluted soil have the capacity to utilize petroleum hydrocarbons and hence could be further stimulated by external nature-based nutrient addition for enhanced decontamination.

4.1.V-09 Organic Matter Decomposition - a New Microbial Laboratory Test Standard

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The soil microbial community is critical in maintaining a healthy ecosystem through processes such as nutrient cycling and organic matter decomposition. Soil microorganisms serve as valuable indicators of soil health, and therefore, their contribution needs to be included within environmental risk assessments. One way to measure changes to soil microbial activity in the presence of contaminants is an assessment of their ability to decompose organic matter. Here, we present a simplified laboratory scale method, derived from the field-based litterbag technique, which can be used to assess the effects of soil contaminants on soil organic matter decomposition or to compare the organic matter decomposition rate among different soils. The organic matter decomposition (OMD) test is based on the mass loss of pre-weighed filter papers, a representative material for organic matter, wherein the mass loss over time is correlated to organic matter decomposition. This method can be applied to most soils including field-collected (reference and contaminated,) or those amended with a chemical substance for risk assessment purposes by which a quantitative measure of toxicity (e.g., EC50) can be derived. The method is currently being validated as part of a new International Organization for Standardization (ISO) standard for Soil Quality: ISO 23265 “Soil Quality – test for estimating organic matter decomposition in contaminated soil”. As part of the standardization process, an international ring test was conducted with the participation of up to 14 laboratories from across Canada, South Korea, and Europe. Our most recent phase involved using LUFA 2.3 soil amended with silver nitrate (AgNO₃), as a representative soil contaminant, along a concentration-response gradient. We will highlight the OMD method along with results from the different phases of the inter-laboratory study, demonstrating the efficacy, validation and standardization of this method. The method contributes to a current gap for the inclusion of microbial studies in risk-based assessments, as well as applicability to derive apical toxicity endpoints.

4.1.V-10 The Influence of Soil Organic Matter Content on the Acute and Chronic Toxicity of Pesticides to *Eisenia andrei*

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Lipophilic pesticides can bind to soil organic matter (OM) reducing their bioavailability to soil invertebrates. Therefore, the risk assessment for lipophilic pesticides (Log K_{ow} > 2) divides toxicity endpoints derived from tests using OECD artificial soil (~10% OM) by a factor of 2 to correct for the lower OM content in agricultural field soils (~5% OM). The application of this assessment factor (AF), however, is questionable, as it is based on results from acute earthworm toxicity tests focussing on mortality, instead of chronic toxicity tests focussing on reproduction. Additionally, the AF assumes that the relationship between bioavailability (and thus toxicity) and OM content is similar for all lipophilic pesticides, which might not be true. To develop a more accurate AF, the relationship between chronic pesticide toxicity and soil OM content for pesticides with different lipophilic characteristics needs to be elucidated. The aim of this study was to investigate the influence of soil OM content on the acute and chronic toxicity of different pesticides to earthworms. Chronic earthworm toxicity tests were performed using *Eisenia andrei*, using growth, mortality, and reproduction as endpoints. Adult earthworms were exposed to five persistent pesticides with a range of lipophilic properties in four soils with different OM contents. Three soils were artificially constructed by mixing quartz sand, kaolinite clay, and varying amounts of sphagnum peat, one soil was a standard agricultural test soil. The data were used to determine LC₅₀ and EC₅₀ values, and the relationships between acute and chronic pesticide toxicity and soil OM content were analysed by linear regression analysis. The results show that acute and chronic pesticide toxicity to earthworms is influenced by soil OM content, and that soil OM influences lethal effects more than sub-lethal effects. Additionally, the results indicate that toxicity-OM relationships differ between pesticides, and that the soil OM content also influences the toxicity of pesticides which are considered non-lipophilic under current legislation (i.e. imidacloprid). Overall, the results from this research will help to provide regulators with a sound basis for increasing the accuracy and ecological relevance of chemical risk assessments.

4.10 Linking soil ecology, ecotoxicology and risk assessment to the impact of multiple stressors on soil organism communities in the field (Poster)

4.10.P-Tu204 MICROSOIL - Correctly Assessing Risks for Microorganisms in Agricultural Soils - Identifying Meaningful Endpoints Under Field-Relevant Pesticide, Biocide and Pharmaceutical Exposures

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Soil health is an important driver for sustainable agriculture and must be maintained and strengthened. For the regulatory risk assessment of plant protection products (PPPs), biocides or pharmaceuticals, effects on the nitrogen transformation (OECD 216) are considered. This process is an important overarching soil function. However, by focussing on one central function of the microbial community, other effects can be overseen. The aim of the project MicroSoil is the identification of meaningful endpoints under field-relevant exposure of PPPs, biocides and pharmaceuticals. It will be investigated whether additional

endpoints relevant for the environmental risk assessment of microorganisms exposed to chemicals in soils should be proposed. By an literature search through various search engines 23 suitable methods were identified covering six methodological groups: Determining (i) the activity of aerobic and anaerobic heterotrophic microorganisms, (ii) nitrifying and denitrifying bacteria, (iii) the activity of exo- and endoenzymes, (iv) the effect on arbuscular mycorrhizal fungi, (v) functional genes and structural changes in soils and (vi) effects on carbon cycling and sequestration. The identified test methods were evaluated according to various criteria such as practicability in the laboratory, costs, replicability/reproducibility and regulatory relevance. For the identification of the most suitable methods, a traffic light system was developed. Based on the evaluation of the test methods, five methods with the highest score for aerobic heterotrophic soil microorganisms (MicroResp™), nitrifiers (ISO 15685), enzyme activity (ISO 20130), arbuscular mycorrhizal fungi (ISO 10832) and structural microbial diversity (ARISA) were proposed as potential test strategy. The sensitivity of the selected test methods will be investigated for six model substances (PPPs, biocides and antibiotics) in three soils varying in their physico-chemical properties. In addition, uncertainties in the risk assessment due to the current consideration of exposure to single substances will also be investigated by determining the influence of repeated applications of e.g. PPPs on processes driven by soil microorganisms. Acknowledgement - The authors thank the German Environment Agency for granting the project (FKZ: 3720 64 411 0).

4.10.P-Tu205 MICROSOIL - Investigation of Alternative Test Methods to Correctly Assess the Impact of Plant Protection Products, Biocides and Pharmaceuticals on Soil Microorganisms

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Agricultural land use profits from a sound relationship between microbes and soil minerals resulting in stable mechanical and fertility properties. For the risk assessment of plant protection products (PPP), biocides and antibiotics only the effects on N-transformation (OECD 216) are considered. This process is an important overarching soil function. However, by focussing on one central function of the microbial community, other effects can be overseen. One part of the project 'Microsoil' is to investigate the sensitivity of three alternative test methods, which can be used to determine effects on nutrient cycles (MicroResp™), enzymatic activities (ISO 20130) and specialists (ammonium oxidizing bacteria, ISO 15685). To gather information about the sensitivity of the methods, the fungicide tebuconazole, the herbicide ethofumesate and the antibiotic tiamulin hydrogen fumarate will be used as model substances besides three other test substances. The tests will be performed in three different soils (LUFA 2.1, RefeSol 02A and RefeSol 04A). LUFA 2.1 with a high amount of sand and a low pH value will be used as worst-case soil. Three nominal test concentrations will be investigated based on the specific predicted environmental concentration. In addition, a control treatment will be prepared. Due to the low solubility in water, the substances will be dissolved in acetone and applied via quartz sand as suggested by OECD 216. At each measurement, the effect on the activity of ammonium oxidizing bacteria, the enzyme activity patterns of arylamidase, arylsulfatase, β -glucosidase, phosphatase and urease as well as the basal and substrate-induced respiration (SIR) will be observed. Measurements will be performed at test start and after 14 and 28 days. If effects above 25% occur, the test duration will be extended up to 100 days. The 1st test with ethofumesate in LUFA 2.1 soil was initiated, using test concentrations of 5.0, 10.0 and 20.0 mg a.s./kg per soil dry weight. The described test methods were performed to observe short-term effects. The results showed that no effect above 25% occurred using the three test systems. Therefore, there is no indication for a short-term effect due to the tested concentrations of ethofumesate in LUFA 2.1 soil. Further data for the other test substances and at different time points will be collected and presented at the conference. Acknowledgement - The study was funded by the German Environment Agency FKZ 3720 64 4110.

4.10.P-Tu206 Effects of Tembotrione Herbicide on the Soil Bacterial, Hppd Communities and on Antibiotic Resistance Indicators: A Microcosm Study

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Soil is known as a (microbial) biodiversity reservoir, whose microbial communities are essential to the functions of this ecosystem. Indeed, microorganisms in soil play an important role in maintenance of ecosystem services, like nutrient cycling, soil structure or crop yield and are recognized as key organisms by the European Union. Herbicides used in agriculture to maintain high yield in crop production, aim to prevent weed growth, but are known to end up in contact with soil microorganisms, that are defined as non-target organisms. One question that microbial ecotoxicologists raise is do the herbicides have toxic effects on soil microorganisms, and does it disturb soil microbial community dynamic and structure? More broadly, short- and long-term impacts on abundance, diversity and functions of these communities need to be investigated. Moreover, an emergent question linked to adaptation behaviors of soil microorganisms exposed to herbicides is the possible co-selection of resistance mechanisms to antimicrobial and to antibiotics. Resistance mechanisms that can thus be shared within microbial communities exposed *via* horizontal gene transfer (HGT). Our study focuses on tembotrione, a recently developed β -triketone herbicide, used in maize crops. This molecule is known to inhibit the 4-HydroxyPhenylPyruvateDioxygenase (4-HPPD) in targeted plants, enzyme also found in numerous soil microorganisms. Thus, our study aims to characterize the potential effects of tembotrione on soil microbial communities, taking also into account the evolution of antibiotic resistance indicators after herbicide application. Soil microcosm approach was performed using 1-fold or 10-fold the recommended agronomical dose of tembotrione, or none for controls, under controlled conditions (temperature and humidity). Soil samples were then collected at day 0 and after 3, 7, 14, 24, 40, and 55 days

to determine the listed endpoints: · The dissipation of the molecule · Viable bacterial abundance with standard culture method, associated to antibiotic resistance analysis · Bacterial diversity of the total bacterial community, and of the *hppd* bacterial community with high throughput sequencing of the 16S rDNA. · Antibiotic resistance genes (ARGs) and HGT genes “dynamics” with PCR and qPCR. Results analyses are still ongoing at the time of writing this abstract but should be available by March 2022.

4.10.P-Tu207 Soil Microbial Composition and Replication Directly Respond to the Presence of Biocides From Building Materials

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Biocides are used in building materials to protect the building against microbial colonization and biodeterioration. However, these biocides are introduced by gradual leaching into soils in proximity of the buildings. The effects of biocides frequently used in facade renders and paints on the soil microbiome are still unknown. Therefore, the study aimed to elucidate the effect of three individual biocides (terbutryn, isoproturone, octylisothiazolinone) and its four combinations of two or three biocides on the bacterial and fungal community composition and proliferation activity. Moreover, soil chemical parameters (soil respiration, pH, anion concentrations, ignition loss), biocide (in realistic concentrations between 0.4 to 6 µg g⁻¹ soil), and biocidal degradation product concentrations were determined at the beginning and after 28 d of incubation and compared to the control (incubation with water). Bromodeoxyuridine (BrdU) immunocapture technique was used in combination with pair-end Illumina sequencing to differentiate the total and active bacterial and fungal microbiomes. In addition, bacterial and fungal gene copy number were quantified via Real-Time PCR analysis. The biocidal treatment did not alter the soil's chemical properties significantly. In turn, the biocidal treatment significantly decreased the total gene copy numbers of both fungal and bacterial community composition. At the same time total bacterial and fungal microbiomes did not significantly change between the biocidal treatments, but the active microbiomes of both bacteria and fungi. While *Desulfotomaculum*, and *Methyloversatilis* decreased, well-known herbicide degraders such as members of the genus *Arthrobacter* increased. Similarly, shifts were found in the fungal community composition, which will be shown in detail by an indicator species analysis. Our results show that biocides as preservatives of façade renders and paints lead to a significant alteration in the active soil microbial community without dramatic shifts in the soil's chemical properties. Therefore, the addition of even low biocide concentration led to a distortion in the activity patterns, which should be characterized with the run-off of façades in future.

4.10.P-Tu208 Measurement and Simulation of Distribution, Degradation, Maximal Soil Concentrations and Ecotoxic Effects of Biocides Being Released From Building Facades

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Biocides are applied to increase the durability of building materials and to protect buildings from microbial colonization and biodegradation. However, biocides are released by gradual leaching and can thus reach soils and ultimately groundwater close to the building. A deeper understanding of this multiparametric process and the development of comprehensive characterization methods remain essential to allow a realistic assessment of the environmental impact of biocides used in building materials. Therefore, facades which formulations contained three in-can preservatives MIT, CMIT and BIT and encapsulated film preservatives zinc phyrithione, terbutryn, OIT as well as zinc oxide in different concentrations in renders and paints were investigated. We conducted two artificial weathering experiments, namely a DIN 16105 laboratory testing (without UV irradiation), and an artificial weathering protocol by a weathering chamber, with controlled UV irradiation. Additionally a real weathering experiment were performed. Biocides and their degradation products were quantified by UHPLC with UV and MS detectors. The observed maximum concentration of BIT was 6.0±0.5 mg per immersion cycle of DIN testing and 5.9±2 mg per rain event of the real weathering test. Eluates were diluted according to local weathering data to reflect realistic dilution by rain, and toxicological effects were evaluated in several soil and sediment toxicity tests for sediment worm, *Lumbriculus* as well as total nitrogen and total carbon transformations. In addition, aquatic toxicity was tested on green algae, daphnia, zebrafish luminescent bacteria, and *Arthrobacter globiformis*. Based on the leaching, weathering, degradation, and adsorption data, the depth-resolved soil distribution was modelled with PELMO considering discontinuous rainfall and biocide input. The predicted biocide concentrations were experimentally validated for different soil horizons. In summary, biocide-containing facade eluates showed toxic effects at early sampling times, but not at later sampling times after application. Biocide distribution in soil was simulated considering discontinuous input and experimentally validated to predict maximum concentration and correlation to acute effects in the ecotoxicological tests. The combination of the prediction of the biocide concentration in soil and further knowledge on the ecotoxicological effects on soil organisms will be presented.

4.10.P-Tu209 Arbuscular Mycorrhizal Fungi and the Need for a Meaningful Regulatory Plant Protection Product Testing Strategy

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Arbuscular mycorrhizal fungi perform key soil ecosystem services and due to their symbiotic relationship with plant roots, may be exposed to the plant protection products (PPPs) applied either to soils or crops. In 2017, the European Food Safety Authority (EFSA) released a Scientific Opinion on Soil Organisms recommending the inclusion of AMF ecotoxicological testing in the PPP regulatory process. In particular, the use of the ISO 10832 spore germination test was proposed. However, it is not clear how this can be implemented in a tiered, robust and ecologically relevant manner. Therefore, a critical review of current literature was conducted to examine the recommendations made within the EFSA Scientific Opinion on Soil Organisms and the methodologies available to integrate AMF into the PPP risk assessment. After assessing potential tier 1 methodologies it was concluded that AMF spore germination testing may be a potential option for the lowest tier of AMF risk assessment, providing that the considerable unknowns, regarding reliability, variability, reproducibility of such lab studies, fungicidal versus fungistatic outcomes, intraspecific diversity and how these study outcomes relate to in-field data, can be appropriately addressed. No recommendations for higher tier studies on AMF were made in the EFSA Scientific Opinion on Soil Organisms. This review concludes that the development of agronomically relevant intermediate and higher tier studies will face significant challenges stemming from the difficulties associated with including an obligate symbiont within the PPP risk assessment, particularly when testing herbicidal products which may be intended to kill the symbiotic plant partner. Finally, the suggestion in the EFSA Scientific Opinion on Soil Organisms proposing that AMF could be protected through the measurement of community metrics such as composition and diversity was considered, but ultimately cautioned against. Instead, there is a need to further the collective understanding of the linkages between the phylogenetic and functional diversity of AMF to ensure any risk assessment scheme is ecologically sound. Overall, this work highlights critical knowledge gaps; significant further research is required to enable the development of relevant, reliable, and robust scientific tests alongside pragmatic and scientifically sound guidance to ensure that any future risk assessment is adequately protective of the ecosystem services it aims to preserve.

4.10.P-Tu210 Chronic Effects of an Insect Growth Regulator - Teflubenzuron - on the Full LIFE Cycle of *Folsomia candida* Via Food Exposure

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Current standardized toxicity tests using soil invertebrates mainly focus on the survival and reproduction of adults. Such results limited to one developmental stage of soil invertebrates are likely insufficient to predict the effects on higher organizational levels. Toxic effects may vary from life-stage to life-stage within the same species. Moreover, the adverse effect may be transferred to the next generation, affecting the offspring but not the exposed adult. The present study evaluates the effects of the insect growth regulator, teflubenzuron (TBF), on the full life cycle of a soil invertebrate aiming to understand the long-term effect of TBF on the life history of springtails. Insect growth regulators such as teflubenzuron inhibit the synthesis of chitin and thereby interfere with the moulting processes. TBF contaminated baker's yeast suspension was provided as the only food source to springtails throughout the experiment. The growth of individual springtails (*Folsomia candida*) was traced from hatching (one day old) and until animals were 2.5 months old. Survival, growth, time to first egg-laying, number of eggs and hatchability were recorded. Concentrations of 4.0, 2.7, 1.8, 1.2, 0.8, 0.5 and 0 mg/L yeast suspension were used. Survival was reduced after two weeks at the high concentrations (4.0, 2.7 and 1.8 mg/L) and reached 0%, 9% and 56% at 40 days. Springtails had good survival at low concentrations, more than 80%, till the end of the experiment. However, negative impacts on growth were detected at all tested concentrations in both juvenile and adult stages. Time to first egg-laying tended to increase as concentrations increased. The accumulated number of eggs was significantly reduced for concentrations of 0.8 mg/L and above compared to controls. The hatching rate was significantly affected at all tested concentrations. Our results show that the toxic effects of TFB are long-term, including slow growth, reduced number of eggs and reduced hatchability. However, such subtle effects are not captured in current standard acute or reproduction tests. Growth and hatchability are more sensitive than survival and they are also important indicators in extrapolating the effects at the organismic level to the population level. Toxicity testing would benefit from the inclusion of such life-history parameters that can be used for mechanistic models to unmask the contribution of growth, reproduction, hatchability to the ecological effects of TFB.

4.10.P-Tu211 Successful Implementation of a Natural Reference Soil Set in OECD 222 Earthworm Reproduction Testing Using Comparable pF Units for Soil Moisture

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According to the current guidelines for ecotoxicological risk assessment of plant protection products for non-target soil organisms, standardized laboratory tests are carried out with artificial soil that meets criteria such as reproducibility and comparability. One of the most used test with standardised artificial substrate is the earthworm reproduction test OECD 222 with *Eisenia fetida/andrei*. Sorption properties like soil organic matter can influence the bioavailability of chemical substances and consequently the toxicity of the exposed organisms. If soils with different properties are not included in the investigations, it can lead to a bias in the results. The artificial OECD substrate has limitations in representing the spectrum of toxicity that could occur in natural soils. To fill this gap, a set of natural agricultural reference soils (RefeSols, Fraunhofer) with a broad spectrum of soil properties that can be found in agricultural soils in mid-latitudes were evaluated in the laboratory with OECD test system 222 and compared to the OECD substrate. A literature review showed that not only the toxicity of some chemicals can vary between natural soils, but also the number of earthworm juveniles produced in the untreated controls can vary greatly between different soil types. Beside food quality, temperature, and pH, soil moisture has a direct influence on the number of juveniles produced. However, the optimal moisture in natural soils for reproduction is poorly understood and previous studies with natural soils and *E.*

fetida/andrei were performed using percentage of WHC_{max} as recommended unit for water content for standard OECD studies. This common method leads to disadvantages in the comparability between soils: the same percentage value of WHC_{max} may mean different actual water availability for the earthworms in different soil types. This is because available water is determined by the soil water matrix potential (often specified as unit pF), a force strongly related to pore sizes and texture. With adjustments in the test performance and the implementation the unit pF, a successful, valid and reproducible implementation of natural agricultural reference soils in the OECD test system 222 is possible. Moreover, mean values for the number of juveniles in the controls of all natural reference soils used were above average when compared to the long-term mean in the OECD 222 substrate.

4.10.P-Tu212 Moisture Level, a Key Parameter in the Earthworm Sensitivity That Is Important to Consider in the Waste and Enrichment Product Risk Assessment. a User Feedbacks

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The guidelines to evaluate the Waste Hazard Property 14 “ecotoxic” (HP14) include test with Earthworms (acute testing (ISO 11268-1) or avoidance testing (ISO 17512-1)), in some European member states. A proposal for a new French regulation on soil enrichment matter include the earthworm reproduction testing (ISO 11268-2) to evaluate the toxicity of enrichment matter before using on soil. Soil moisture is a decisive factor for earthworm survival and growth. Studies suggest that earthworm’s sensitivity and behavior can be influenced by the moisture level in the soils. Moreover, the earthworms used in normalized guidance are not typical soil species and few studies have been performed to characterize the moisture requirements of *Eisenia fetida* in soil or other substrate. According to the wide range of waste and enrichment matter and their influence on soil holding water, it is crucial to better understand the effect of moisture level on the sensitivity of earthworm. Although documented in the test guidelines as a rate of the maximum holding water, these guidelines don’t consider the very specific characteristics of waste and soil enrichment matters. In this study, the sensitivity of *Eisenia fetida* was evaluated at different moisture levels. For example, the mortality of worms was measured with 3g/l of boric acid in soils at different water contents. The result demonstrates a linear relationship between the moisture level and the mortality: below 50% of water holding capacity, 100% of worms died, whereas over 70% of water holding capacity, only 10% of worms died. This study is a user feedback on the earthworm ecotox testing in the waste and enrichment product risk assessment context and a contribution for a better use of earthworm in ecotox lab testing.

4.10.P-Tu214 Towards a Toxicokinetic-Toxicodynamic Model for Earthworms: Integrating Movement and LIFE Histories

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The environmental risk assessment of plant protection products aims at the prevention of any adverse effects on the environment including soil health. Therefore, the risk assessment for in-soil organisms, such as earthworms, is based on two key elements: the effect assessment and the exposure assessment. In the current regulatory frameworks toxicity endpoints and predicted environmental concentrations are compared to derive risk quotients for the characterization of risks. This procedure, however, largely ignores that environmental exposure concentrations are variable in space and time, and the exposure of moving organisms will vary in time too. In turn, the movement of earthworms can be affected by a number of abiotic factors as is their growth and reproduction. These factors include the moisture level, temperature, and the organic matter content. In laboratory experiments, earthworms showed a decrease in burrowing activity with decreasing moisture levels and even avoidance behavior of these unfavorable moisture environments. Furthermore, experimental results indicate that earthworm movement ceases completely at low moisture levels. At these conditions, individuals enter an inactive state, also described as diapause or aestivation. Moreover, the burrowing activity of earthworms is affected by temperature, for which the number of casts produced per earthworm was used as a proxy in laboratory experiments. Finally, the organic matter content plays a significant role for the burrowing and movement of earthworms. In this poster, we present a Dynamic Energy Budget model for the endogeic earthworm *Aporrectodea caliginosa* to integrate the effect of the abiotic factors on earthworm movement and life-history. This development is a major step towards a toxicokinetic-toxicodynamic model for earthworms.

4.10.P-Tu215 Phytoremediation of Pesticide Contaminated Soils and the Transformation of Chlorinated Pesticides in PLANTS

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Soil is presumed a risk matrix in terms of the transfer of pollutants into the food chain. Pesticide residues in the soil are of big interest in agricultural pollution. Due to their properties, such as slow degradation and accumulation in living organisms, the removal of chlorinated pesticide (OCP) residues in the soil is considered pivotal. Knowing the limitations of conventional treatment such as high cost or the disturbance of soil flora and fauna, phytoremediation is assessed as a sustainable approach for soil reclamation. The identified fundamental processes of the plants used for remediation make this environmental friendly clean-up method a promising approach for OCP contaminated soil. This study’s goal is to establish a phytoremediation method for OCP contaminated farmland by an enhanced transformation of OCPs accumulated in plants and aims to evaluate the ability of different adjuvants such as distinct fungi (i.e. white rot or brown rot fungi) and of the use of mobilizing agents to boost phytoremediation efficiency. First experiments for the optimization of the OCP uptake from contaminated soil into the plant are being conducted in greenhouse experiments by closely observing plant health parameters. By this, a suitable, strong growing plant with the ability for OCP uptake should be identified. The study design of the first experiment contained different replicates of contaminated and uncontaminated soil and the studied plants were *Miscanthus x giganteus* and *Ricinus communis* L. The first preliminary results show that both studied plants have the capability for OCP uptake. These first results are being used for planning the next

greenhouse research, during which mobilizing agents will be added and the increase of OCP uptake will be evaluated. Afterwards, the fate of OCP accumulated in plants under conditions in the fermenter will be extensively studied in laboratory experiments with sophisticated analytical methods (GC-MS and LC-HRMS) with regard to emerging transformation products.

4.10.P-Tu216 Genome Sequencing Reveals Active Functionalities in Petroleum Degrading Consortium From Impacted Soil

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Crude oil pollution is a major environmental problem in the Niger Delta region, Nigeria which has destroyed both biodiversity and ecosystem services severely. Functional attributes of a petroleum degrading consortium developed from bacteria isolated from crude oil-polluted soil with 4923-51780 mg/kg of total petroleum hydrocarbons (TPH) in Igbuduya, Niger Delta region, were investigated using deep sequencing to ascertain the site's amenability to nature-based reclamation. Enrichment in 1% crude oil- and naphthalene-Bushnell Haas (BH) broth was used to select for isolates with enhanced degrading potentials. Axenic cultures that decolorized 1% 2,6-Dichlorophenolindophenol (DCPIP) redox dye in hydrocarbon-BH broth degradation assay within 24h were sub-cultured and extracted genomic DNA was further analyzed with the ZymoBIOMICS shotgun metagenomic sequencing for microbiome analysis. Sequencing libraries were prepared with the KAPA HyperPlus library preparation kit while final library was sequenced on the Illumina NovaSeq platform. Genome analysis by composition barplots at strain level revealed the presence of *Micrococcus luteus* (83.54%), *Micrococcus aleoverae* (14.64%), *Micrococcus luteus* Mu201 (0.72%) and *Sphingobium barthaii* (0.1%). Functional pathways for nucleotide biosynthesis and hydrocarbon degradation very prominent and dominant in the consortium were most affiliated with *M. luteus*. This deep sequencing insight established that the indigenous microbes in the oil-impacted site have the genetic capacity to obtain carbon and energy sources from the contaminant during biodegradation and thus could be further stimulated for enhanced bioremediation of the polluted site.

4.10.P-Tu217 Agricultural Waste Recycling, Sustainable Food Production and Ecosystem Services: Circularity in a Case Study of Tomato Production

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In the mid-20th century, advances in scientific knowledge helped the development of the Green Revolution, characterized by a fast increase of agricultural production, essential for a flourishing world population. Still, this intensification in agricultural production has been responsible for ecosystem deterioration, which is imperilling the delivery of ecosystem services. As global food demands continue to grow, crop yields show signs of stagnation adding further pressure to agriculture. A new revolution is urgent to ensure food security, one that is rooted in novel sustainability paradigms such as efficiency and circularity. Technological innovations focus on the use, transformation, and valorisation of agroindustrial wastes into innovative products aimed to ameliorate soil properties, reducing fertilizer dependence, and maintaining ecosystems integrity. Special attention is being given to integrated strategies that promote nutrients' circularity, such as agro-waste based fertilizers (AWBF). The present study used an ecosystem services approach to evaluate the effectiveness and environmental impact of an AWBF derived from the digestion of vegetable residues by *Hermetia illucens* larvae (product at the developmental stage). In a greenhouse, a randomized block experiment was set with four fertilization treatments: 1) reference (no fertilization), 2) NPK plus horse manure (conventional fertilization), 3) NPK plus AWBF, and 4) AWBF alone. An integrated assessment included: tomato productivity and quality (provisioning services), soil biodiversity (regulating services), functionality of microbial communities (supporting services), *in situ* feeding activity of soil organisms (supporting services) and soil retention function (regulating services). Overall, the incorporation of AWBF, alone or in combination, did not impact supporting services, such as the activity of extracellular soil enzymes involved in nutrient cycling (dehydrogenase, urease, alkaline and acid phosphatase, aryl sulfatase and beta-glucosidase), and feeding activity of soil organisms; still, low consumption rate was observed in all treatments. In addition, soil elutriates did not impact the growth of *Raphidocellis subcapitata* and *Lemna minor*, nor *Daphnia magna* mobility. This holistic perspective demonstrated that AWBF can be considered an adequate alternative to conventional fertilization treatments, which may improve the sustainability of ecosystems.

4.10.P-Tu218 Triticum Aestivum Bioaccumulation and Physiological Responses to Copper Nano-Formulations and Ionic Copper: An Indoor Mesocosms Approach

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Nano-based copper (Cu) formulations are getting more attention in the recent years. Key property of these novel pesticides is a gradual release of Cu ions resulting in higher efficacy than in the case of conventional formulations. Lower application rates of nano-pesticides may bring economic and environmental benefits. Yet, to assure sustainability, it is crucial to understand their behaviour, fate, and toxicity in soil ecosystems. We performed an indoor mesocosms study to understand how nano and non-nano Cu formulations affect soil functions and health, while assessing their bioavailability and bioaccumulation potential. A model

community in each soil core contained: wheat *Triticum aestivum*, earthworms *Eisenia andrei*, isopods *Porcellionides pruinosus*, mealworms *Tenebrio molitor*, plus soil microbiome. Four compounds were tested, at 50 mg Cu/kg of LUFA 2.2 soil: ionic form as $\text{Cu}(\text{OH})_2$, and nano-formulations - Kocide 3000®, $\text{nCu}(\text{OH})_2$, and nCuO . LUFA 2.2 represented a negative control. Destructive sampling was conducted in triplicates, on day 14 and 28 (and day 0 and 6 for plant bioaccumulation), collecting the soil for microbiome analysis, Cu quantification (dissolved and total), and the organisms for biomass and biomarker assessment. Part of this work addressing plant responses is presented here. We hypothesized that plant bioaccumulation patterns and physiological responses depended on the Cu form. $\text{Cu}(\text{OH})_2$ had the highest effects on root and shoot fresh weight, and shoot length. No statistically significant differences were found for the nano-formulations. Effects of $\text{Cu}(\text{OH})_2$ on biomass were more pronounced in the aerial part. For all the treatments, total Cu concentrations in the roots were 10-fold those in the aerial part. The highest Cu content was in the roots exposed to $\text{Cu}(\text{OH})_2$. Cu toxicokinetics for the roots and for the aerial part was described using the classic one-compartment model. The highest uptake rate constant in the roots was observed for $\text{Cu}(\text{OH})_2$. The uptake pattern was similar among nano-formulations. Substantially lower uptake rate was observed in all the treatments for the aerial part. Pigment contents did not differ, and there was no treatment-induced lipid peroxidation. Total phenolic content in *T. aestivum* shoot was significantly increased as response to $\text{Cu}(\text{OH})_2$. The largest overall impact of the ionic Cu form in this study is aligned with the higher bioavailability observed during the experiment compared to nano-formulations.

4.10.P-Tu219 Response of Ginger Growth and Soil Phosphorus Availability to the Different Years of Chloropicrin Fumigation

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Although chloropicrin (CP) fumigation is widely used to control soil-borne pathogens, it can also change the cycle of soil nutrients by affecting soil beneficial functional microorganisms. However, the effect of repeated use of CP fumigation on soil phosphorus (P) availability is still largely unclear, which is the main problem we want to solve in this study. A field experiment was conducted in ginger fields with 0, 3 and 7 years (F0, F3 and F7, 3 replicates of each) of annual CP fumigation history in Shandong Province of China. Soil samples (0-20 cm) were collected at four different times in 2019: Before fumigation (05/03/2019), After fumigation (28/03/2019), Middle growth period (24/08/2019) and Harvest (23/10/2019). Ginger yield, soil phosphatase (acid and alkaline) activity and soil P fractions from different sampling periods were measured and compared with three experimental replicated plots. Results showed that ginger rhizome yield was similar in F0 and F3 (70.0 t ha⁻¹), but significantly lower in F7 (37.5 t ha⁻¹). The acid phosphatase (AiP) activity was significantly higher in F0, while alkaline phosphatase (AIP) activity was the highest in F3. There was no significant difference in the available P (Resin-P+NaHCO₃-P+NaOH-P) between F0 and F7, with 33.6% to 57.5% of total P (TP), while the available P was significantly lower in F3, being less than 30% of TP. Redundancy analysis (RDA) showed that pH values were significantly negatively, while AiP activity was significantly positively, correlated with the proportions of soil available P to total P. The results showed, although the yield of ginger rhizomes was similar between F0 and F3, the significantly lower proportion of soil available P in F3 may be a key limiting factor for higher ginger yield. After 7 years of repeated CP fumigation, soil-borne pathogens may be resistant to CP fumigation. At this time, the serious death of ginger has become the main limiting factor for ginger production, and we should first consider finding another effective method instead of sole CP fumigation to prevent soil-borne diseases. The high proportion of soil labile P fractions in F7 may further cause serious environmental problem such as water eutrophication. Decreased phosphatase activity after repeated CP fumigation is also evidence of reduced soil P availability, which highlights the necessity of further research on soil P solubilizing microorganisms to reveal the microbial mechanism.

4.10.P-Tu220 Response of Soil Phosphorus Availability and Ginger P Uptake to the Combined Application of Chloropicrin and Azoxystrobin

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Although chloropicrin (CP) and azoxystrobin (AZO) are widely used to control soil-borne pathogens, they can also change the cycle of soil nutrients by affecting soil beneficial microbes. However, the effect of the combined application of CP and AZO on soil phosphorus (P) availability and plant P uptake is still largely unclear, which is the main problem we want to solve in this study. A 28-week greenhouse experiment was set up with six treatments: CK (Control), AZO1 (A single application of AZO), AZO2 (Two applications of AZO), CP (CP fumigated with no AZO), CP+AZO1 (CP combined with AZO1) and CP+AZO2 (CP combined with AZO2). Ginger was used as the model plant. Field recommended amounts of 37.1 g m⁻² of CP and 47.1 mg m⁻² of AZO were used for each application. Soil was sampled four times at different growth period and ginger plants were sampled at harvest. Basic soil chemical properties, phosphatases activity, soil P fractions, and ginger P uptake were tested. Results showed that soil acid (AiP) and alkaline (AIP) phosphatase activity were significantly lower in CP-treated soil than in CK during the entire growth period, while AZO did not have significant inhibitory effects on soil phosphatases activity until 17 weeks after plant (WAP). The proportions of labile P (Resin-P, NaHCO₃-Pi and NaHCO₃-Po) to total P (TP) were significantly higher in AZO1 and CP (29.3% to 35.5% of TP) than in CK (20.5% of TP) at 9 WAP. Only CP-treated soil had significantly higher proportions of labile P (31.3% to 36.5% of TP) than CK (21% of TP) at 17 WAP. Soil inorganic P fractions and organic P (NaHCO₃-Po) fractions were highly negatively correlated with soil pH and phosphatases activity, respectively. The height and total P content of ginger plant were significantly higher in CP (height 86.0 cm, total P content: 43.6 mg) and CP+AZO2 (height 72.1 cm, total P content: 42.0 mg) as compared to CK (height 53.2 cm, total P content: 20.0 mg). The rhizome P content was significantly highly

in CP+AZO2 (18.3 mg) than in CK (6.1 mg). The ginger physiological P use efficiency decreased from CK (1799.8 g DM g⁻¹P) to CP+AZO2 (589.0 g DM g⁻¹P). From the result, we could conclude that CP fumigation increased the proportions of soil in organic P proportions and ginger total P uptake, but no synergistic effects were observed after the combined application of CP and AZO. Therefore, ginger planting farmers may avoid the unnecessary AZO application in soils with CP fumigation.

4.10.P-Tu221 Evaluating the Compounded Effect of Environmental Disturbances and Pesticide Mixtures on Soil Microbes

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Pesticides are widely used in conventional agriculture, either applied separately or in combination, and often in serial application during the culture cycle. Due to their occurrence and persistence in soils, pesticides may have an impact on soil microbial communities and on supported ecosystem services. In this regard, the EFSA (European Food Safety Authority) recently published a scientific opinion inciting to include more soil microbe-mediated processes in pesticide risk assessment. Climate change is another major concern for microbial community stability. Extreme climatic events, such as droughts or heavy rainfalls, are becoming more and more frequent and their impact on soil microbial diversity and functioning have already been demonstrated. The objective of this study is, therefore, to evaluate the compounded effect of global change related environmental disturbances and pesticides on soil microbial community structure and functioning. To this end, 270 soil microcosms were exposed to either a heat disturbance, heavy rainfall conditions, or no environmental disturbance (control). After a 3 days recovery period, the active ingredients clopyralid, cypermethrin and pyraclostrobin that are commonly used as herbicide, insecticide and fungicide, respectively, were applied either in single application or combined at 1X or 10X of the agronomical application rate. Control and treated microcosms were then kept under controlled conditions for 42 days. The ecotoxicological impact of pesticides' application scenario conditionally to the prior environmental disturbance will be evaluated on total bacterial community diversity and structure using sequencing of 16S rDNA gene amplicons. We will focus specifically on the effects of treatments on the abundance of ammonia-oxidizing archaea and bacteria via q-PCR, and on the subsequent changes in mineral nitrogen pools. We hypothesize that climate change related environmental disturbances and pesticides' mixtures application may have compounded effects on the structure and functioning of some key microbial community members as compared to single pesticide applications. The results from this work will provide new knowledge and insights for the scientific community and may help fill the regulatory gaps on pesticide risk assessment.

4.10.P-Tu222 Chronic Copper Exposure Shifts the Thermal Performance Curves and Temperature Preference in the Springtail *Folsomia candida*

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Toxic effects of chronic exposure to low-dose contaminants in animals has been continuously drawing attention due to their occurrence and persistence in the soil of urban, rural, industrial, and natural environments. The aim of this study was to investigate the chronic effects of copper contamination on thermal performance and behavioural temperature preference in a typical soil invertebrate, *Folsomia candida*. We hypothesize that bioaccumulation of copper alters the position and shape of thermal performance curve and changes temperature preference in springtails. Soils contaminated with three copper levels (approx. 40, 400 and 1500 mg/kg dry weight soil) were collected from a former timber preservation industry at Hygum, Denmark. Age-synchronized springtails (12-14 d) were exposed to the three exposure levels in Hygum soil and distributed to 10 temperatures (0, 2.5, 5, 10, 15, 20, 22, 24, 26 and 30 °C) with 6 replicates (n=10). After a three-week exposure, springtails were weighed and the numbers of the animals and their offspring were counted to draw thermal performance curves regarding survival, growth and reproduction. A temperature preference experiment was conducted using rectangular arenas (75.8 cm length × 4.7 cm width) with a temperature gradient from approximately 5 to 35 °C. After copper-exposure for 3 weeks, 50 springtails were placed in the middle of the panel (approx. 20 °C) with and without temperature gradient, respectively. The distribution of animals was recorded every 10 minutes in the total period of 1 hour in order to estimate the temperature preference distribution. The results of this research demonstrated the toxic effects of copper contaminations on thermal performance and temperature preference in springtails, which is essential for evaluating the individual plasticity and population resilience as well as their ecological function and service.

4.10.P-Tu223 The Response of Earthworm *Eisenia fetida* to Triclosan Exposure Under Drought Stress

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Antimicrobials are essential for the maintenance of public health and life quality. Triclosan (TCS), widely used as an antimicrobial agent, is usually introduced into soil by sewage sludge application and may present potential risks in the terrestrial ecosystem. Recently, large numbers of studies carried out on TCS-induced physiological and biochemical effects on soil biota. Nevertheless, these studies did not consider the co-occurrence of different stress sources in soils related to ongoing global warming. Changing climate conditions might alter both physical and chemical soil properties. Due to higher temperatures and increasing evapotranspiration rates, a general decrease in soil moisture content is expected. This threatens many soil organisms that rely on these moist environments for their survival as well as is likely to change the toxicity of various soil pollutants. Earthworms *Eisenia fetida* are directly exposed to soil pollutants with their thin epithelium and their feeding, thus, they are highly relevant for the assessment of the toxicity of inorganic and organic contaminants to soil biota. The main objective of this study was to evaluate the influence of decreased soil moisture on TCS-induced changes in *E. fetida* growth, mortality, and oxidative stress levels. 4-weeks triclosan-contaminated soil (10 - 750 mg TCS kg⁻¹) experiments with *E. fetida* were performed at different

soil moisture contents (60% and 30% of the soil volumetric content). Earthworm growth, survival rate, biochemical indicators (the activity of enzymes) and the damage of oxidative stress (lipid peroxidation) were detected.

4.10.P-Tu224 Effects of Increased Atmospheric CO₂ Levels With WATER Stress Situations on the Avoidance Behaviour of Soil Invertebrates Towards Metal(Loid)-Contaminated Soils

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Across the globe, different areas are known to be affected by anthropogenic metal(loid) contamination. In addition, climate changes are leading to increased atmospheric CO₂ levels and air temperature along with variations in the precipitation patterns, with a higher incidence-severity of extreme events such as droughts and floods. In the terrestrial environment, soil organisms are surrounded by a complex matrix, in which climate factors can modify the bioavailability of metal(loid)s, due to changes in some edaphic parameters, but also alter the performance of soil invertebrates and/or their susceptibility to contaminants. Although the effects of increased temperature have been explored, the possible impacts of rising atmospheric CO₂ levels have received less attention. Considering all the above mentioned, the aim of the present study was to evaluate the effects of combining high atmospheric CO₂ levels with water stress situations on the avoidance behaviour of the invertebrate species *Enchytraeus crypticus* towards metal(loid)-contaminated soils. With that purpose, two historically metal(loid)-contaminated soils were collected in central-northern Portugal: a soil from a former agricultural area near an industrial chemical complex, and a soil from a former metal mining district. Avoidance tests with *E. crypticus* were performed under different climate scenarios based on the IPCC predictions for 2100: 1) standard conditions – climate conditions recommended by OECD guidelines (20 °C + 50% soil water holding capacity -WHC- + 400-500 ppm atm. CO₂); 2) high atmospheric CO₂ levels with soil drought conditions (20 °C + 25% soil WHC + 1000 ppm atm. CO₂); 3) high atmospheric CO₂ levels with soil flood conditions (20 °C + 25% soil WHC + 1000 ppm atm. CO₂). As previously observed for standard conditions, *E. crypticus* significantly avoided the metal(loid)-contaminated soils tested. Though, when exposed to high atmospheric CO₂ levels combined with water stress situations, enchytraeids lost their ability to avoid metal(loid) contamination. This pattern was clearer in the drought scenario probably due to the susceptibility of these soft-bodied oligochaetes to desiccation. Understanding the response of soil invertebrates in the given context is of utmost relevance since multiple stress conditions are the most common situations for natural populations.

4.10.P-Tu225 The WARMET Project: An Interdisciplinary Approach to Evaluate the Response to Climate Change of Abandoned Mine Wastes Colonized by Vegetation vs. Restored in Semiarid Areas

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WARMET is a research project funded by the Spanish Ministry of Science and Innovation (PID2020-118941RA-I00) that began in September 2021 and will last for three years. Metal mine wastes are often deposited in open-air piles (mine tailings). Classic-restoration approaches (technical reclamation) usually involve topsoil capping aiming to create a new soil above wastes and then afforestation. These options have been questioned in semiarid regions due to ecosystem inability to be self-sustaining. An alternative is to promote the growth of plants in mine wastes (phytomanagement by phytostabilization). This contributes to create vegetated patches that may promote plant-soil feedbacks and ecosystem functioning. If tailings pose immediate risks, the necessary measures should be made available for immediate technical restoration. However, if conditions are not so extreme, the spontaneous succession of plants directly growing in wastes has some advantages (e.g., plants adaptation to local climate, improved long-term sustainability). Previous works showed that the direct grow of native plants in tailings can provide functional soil-vegetation systems with attenuated ecotoxicity, but functional and ecotoxicological aspects of conventionally restored tailings are scarce or even lack. Furthermore, how systems will respond to climate change is still an open question. Answering these questions is core for evaluating the suitability of phytomanagement vs. conventional techniques for mine tailings restoration in semiarid regions. WARMET aims to assess whether spontaneously-colonized and conventional technically-reclaimed (capping and afforestation) metal mine tailings soils of Mediterranean semiarid regions differ in their response to climate change in terms of functionality and ecotoxicity. To achieve this an interdisciplinary study is being carried out in tailings from SE Spain: i) field work (evaluation of the functionality and ecotoxicity of the soil-plant system from different types of mine tailings, considering areas with vegetation and adjacent bare soils); ii) lab-experimental work (subjecting tailing soils to different climate change scenarios according to IPCC predictions). Physical, physico-chemical and biological soil parameters related to systems functionality and ecotoxicity are being considered. Soil ecotoxicity is being assessed through a battery of bioassays (microorganisms, invertebrates and plants). The poster presents the outline of the project.

4.10.P-Tu226 Combined Effects of Chlorpyrifos and Microplastics Derived From Conventional and Biodegradable Mulch Film on Earthworm (*Lumbricus terrestris*) and Their Fate in Soil

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In this study, we investigated the combined effects of low-density polyethylene (LDPE-MP) and PBAT/PLA (Bio-MP)

microplastics, and insecticide chlorpyrifos (CPF) on growth, reproduction, and survival of earthworm *Lumbricus terrestris*, and the transport of microplastics, chlorpyrifos and its metabolites by earthworm activity in 60-day mesocosm experiment. Results showed that Bio-MP strongly inhibited earthworm growth and survival rate while the reproduction of earthworms was not affected by none of the microplastics. The combined effects of microplastic and chlorpyrifos on earthworm depended on the type of microplastic and their exposure concentration. CPF facilitated the transport of LDPE-MP while inhibited the transport of Bio-MP into the soil by earthworms. LDPE-MP facilitated the assimilation of CPF by earthworms while Bio-MP decreased it at 1250 g. a.i./ha CPF application rate. LDPE-MP did not influence the transport of CPF by earthworms into the soil while Bio-MP enhanced the CPF transportation. 3,5,6-Trichloropyridinol (TCP) was the main metabolite found in this study and the effects of microplastics on the TCP concentration in the earthworm bodies followed that of microplastics on CPF concentration in the earthworm bodies. Both LDPE-MP and Bio-MP decreased the TCP concentration in soil indicating that microplastic could extend the degradation half-life of CPF in soil. Therefore, we suggested that the type and exposure concentration of microplastic are important factors influencing the combined effects between microplastics and other pollutants, and their interaction effects should be considered when studying their fate in soil ecosystem.

4.10.P-Tu227 Uncertainty Analysis of the NOEC Estimation for Count Data Sets Based on Field Data and Virtual Experiments

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In terrestrial field experiments and aquatic mesocosm studies, the no observed effect concentration (NOEC) is in most cases an important endpoint. Therefore, identifying the most appropriate hypotheses test with the highest statistical power is an advantage. This is especially true when multiple testing approaches are used and the so-called alpha inflation has to be managed. The most common methods are the correction of the respective values from the t-tables or a correction of the alpha values itself (e.g. as in Holm- or Bonferroni-correction). In general, the preferred parametric tests have a higher power compared to non-parametric tests, but assume that data are (approximately) normally distributed and the variances are homogenous. In case of abundance or reproduction data sets, which consist of count data, a Poisson distribution is the correct underlying distribution. Additionally, especially for field tests, the sample size is low and variance structure heteroscedastic. Currently, the well-known t-tests of Dunnett and Williams are used to derive NOEC estimations for field tests or mesocosm studies. However, such multiple t-tests do not work well for count data due to the missing normal distribution and variance heterogeneity. In case of the Dunnett test, this can be compensated by combination with e.g. Generalized Linear Models and Generalized Linear Mixed Effects Models. By contrast, non-parametric tests as the U- test or the Jonkheer Terpstra tests display low power compared to the tests of the t- test family. Lehmann et al. (2015, 2018) developed the closure principle computational approach test (CPCAT) to address Poisson distributed data. As a major advantage, CPCAT does not require normal distribution or variance homogeneity and alpha inflation is avoided by the closure principle. Instead, the true Poisson distribution of the data is used as a basis for testing. We examined the practicability of CPCAT regarding derived NOEC-values when compared to those obtained using Dunnett (in combination with different models), Williams t-tests and non-parametric tests in a virtual experiment based on field data. The main goal was to investigate whether endpoints derived with CPCAT would be at least as protective as NOECs derived with other tests and how different scenarios would affect the outcome of the analysis regarding data variance. Numerical scenarios will be presented, covering different variance structures and effect levels in the treatments.

4.10.P-Tu228 New Tools for Predicting Environmental Concentrations in Soil in Regulatory Context - Potential Implications for the Soil Organism Risk Assessment of Plant Protection Products

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The EU risk assessment (RA) for soil organisms exposed to plant protection products is conducted as a two-tiered approach. At Tier 1, laboratory derived ecotoxicity endpoints are compared to predicted environmental concentrations in soil (PECsoil). If a potential risk is indicated, higher tier field studies as the only option can investigate whether effects on soil organism communities occur at relevant field application rates under natural conditions. We present results of an impact assessment investigating how the new EFSA guidance for PECsoil calculation published in 2017 may impact the soil RA in the future. More than fifty active substances and metabolites have been assessed. For the ecotoxicological RA, the PECsoil was divided by the regulatory acceptable concentration (RAC) for soil organisms based on EU agreed endpoints to calculate the failure rate (compounds with PEC/RAC ratio > 1). We focused on impacts of the new exposure framework on soil RA and discuss its applicability within the current EU RA scheme. Detailed modelling results are discussed in the poster of Multsch et al. We observed a considerable increase of PECsoil values for active substances (compared to current modelling) which differs between regulatory zones in the EU: Across all zones, elevated PECsoil-values (based on total concentrations) increased RA failure rates by up to 67%. Compared to the current failure rate of 14%, up to 5 times more field studies will be triggered if new modelling is implemented at Member State level. Yet, it is unclear how to translate exposure values from the new modelling tiers to ecotoxicological endpoints from either lab or field. Selection procedures of geographic locations for exposure assessment results in incompatible soil properties (e.g., organic carbon content, bulk density) between e-fate and ecotoxicological data. This inconsistency hinders scientifically reasonable comparison and interpretation. At present, the new modelling framework should not be used before the availability of

an updated soil organism RA guidance which specifies how these exposure values should be implemented in a tiered RA approach. Ecotoxicological ‘intermediate-tier’ effect studies or modelling approaches may be a potential option to overcome such discrepancies in the future but are currently not available/accepted. A sufficiently long transition period will be required to ensure alignment between the new modelling framework and the tiered assessment for soil organisms.

4.10.P-Tu229 Do We Know Enough About Pesticide Impacts on Soil LIFE in Southern Africa, Given Declining Soil Quality and Food Security?

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Southern Africa is characterized by ‘old soils’, of limited quality. In the framework of climate change maintaining good quality, soils become more important. Soil organisms play an indispensable role in maintaining and improving soil quality. Therefore, we must know to what extent pesticides, used in large and small scale agricultural systems in southern Africa could affect the abundances and activities of soil organisms. To this end, we did a bibliometric inventory of CAB and Scopus databases for relevant research papers. The resulting numbers of research papers are limited and cover only a few soil species (mainly earthworm species) and limited numbers of pesticides (*viz.* Cu-oxychloride, chlorpyrifos, azinphos-methyl, carbofuran, mancozeb). The results show that these pesticides applied under field conditions can damage soil life. Data about the impacts of the most used pesticides in southern Africa are for the greater part lacking, however. Moreover, the knowledge about soil fauna species typical for SSA soils is almost absent, in particular, the soils of poor quality under dry environmental conditions. Therefore, there is a need to fill these data gaps with research on other soil species and pesticides. It is a question then, whether it is possible to rely on research carried out in other parts of the world and use bridging factors? Or is additional research in southern Africa needed?

4.10.P-Tu230 Inventory Analysis of the Contamination of Agricultural Land With Biocides, Pharmaceuticals and Plant Protection Products in Germany

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Agricultural soils in Germany are exposed to a variety of substances. However, the overall residue contamination situation is unknown. Several substances are applied directly or indirectly to soils and can accumulate there - depending on the respective soil and substance properties. Published studies on agricultural soil pollution in Germany are limited and often restricted to single (often well known) substances or substance groups. Organisms living in such soils are exposed to numerous chemicals from different uses over a long period of time. However, the risk assessment for active substances of plant protection products (ppp), biocides and pharmaceuticals as well as industrial chemicals under the different European regulations does not consider multiple exposure of different substances. This has already been identified as one of the major deficits of the single substance assessment in the approval procedures of ppp (Topping et al., 2020; Frische et al., 2018), but is also common practice in the approval procedures of biocides and pharmaceuticals. So far, it is unclear to what extent multiple exposure is relevant in the field situation and whether neglecting the realistic exposure leads to an underestimation of resulting risks in the individual approval procedures of chemicals. In 2021, a project funded by the Federal Ministry for Environment, Nature Conservation and Nuclear Safety was initiated, with the aim of obtaining a first screening of the pollution level of agricultural soils in Germany together with the investigation of their biological conditions. Knowledge of the current contamination situation of agricultural soils is necessary to check the plausibility of models for regulatory substance approval. Moreover, current assumptions underlying risk assessment schemes for soil organisms can be checked against the situation in the field. An overview of the actual contamination situation in connection with a status survey of the soil organism community can therefore provide a valuable contribution to the further development of risk assessment of pharmaceuticals, biocides, ppp and industrial chemicals and can significantly influence approval decisions in the future.

4.10.P-Tu231 Effects of Pesticides on Earthworms and the Soil Microbiome in Agricultural Soils Across Europe. a Peek on Results From the SPRINT Project

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Recent research has shown that contamination of European agricultural soils with residues of plant protection products (PPPs) is not the exception but is rather the rule. Studies have shown that over 80% of analysed samples contain residues of PPPs, 58% mixtures of up to 13 different substances. Even in soils under organic management up to 5 PPP residues have been detected. Additionally, an investigation has found higher concentrations of currently used pesticides (CUPs) in earthworms compared to soils. Much is still unknown about the impacts of these residues in the environment, especially when mixed – often described as a ‘cocktail effect’. The H2020 SPRINT (Sustainable plant protection transition) project intends to determine the influence of PPPs on human health and the environment. Therefore, 10 case study sites (CSS) across Europe and one in Argentina, covering the main cropping systems, are studied. Each CSS contains 10 fields under conventional and 10 fields under organic management. In this study we focus on the terrestrial environment including soil, earthworms and soil microbiome. Collection of the environmental samples (*e.g.* soil and earthworms) took place in all CSS during the growing season of 2021, after 50 - 80% of

pesticides have been applied. We studied the effects of pesticide use as well as multiple pesticide on earthworm populations and the soil microbiome. Furthermore, we correlate these effects to the cropping system and the management practice. Our results show the effects of multiple pesticide residues under different conditions on the soil ecosystem. As a result, we will present bioindicators that are sensible to multiple pesticide residues. These data can be used as a base for the development of new EFSA guidelines regarding tests for pesticide effects on the terrestrial environment.

4.10.P-Tu232 Towards a Better Protection of Soil - the PROSOIL Project & the Development of Soil Toxicity Criteria for the CLP Regulation

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During the environmental hazard assessment for the soil compartment, ecotoxicity data on various organisms is generated and used in the context of different European substance legislations (e.g. REACH, pesticides, biocides, or pharmaceuticals). In order to categorize and better communicate the hazards of substances, the European Classification, Labelling and Packaging regulation (CLP) together with the international Globally Harmonised System (GHS) are central and overarching instruments across legislations. Despite the availability of soil ecotoxicity data, classification and labelling within CLP/GHS is currently based on aquatic toxicity criteria, i.e. defined regulatory threshold concentrations, solely. In the CLP context, it is assumed that existing criteria for aquatic organisms are conservative enough to sufficiently protect and inform on hazards for terrestrial organisms. Around 15 years ago, historic initiatives and proposals aiming at establishing terrestrial toxicity criteria for classification and labelling failed at the EU and UN level. Meanwhile, more ecotoxicity data became available. Building upon these developments, the PROSOIL project aimed at deriving toxicity criteria for soil organisms on a comprehensive data basis. As a first step, a comprehensive harmonised database of available ecotoxicity data for various soil organisms was compiled from regulatory repositories. The project focused on terrestrial organism groups potentially exposed to toxic substances via soil, i.e., plants, invertebrates (e.g., oligochaetes, collembolans, mites), and micro-organisms. Above-ground organisms such as bees, birds, or mammals were not considered. Secondly, toxicity ranges for different soil organism groups were analysed and possible toxicity thresholds were identified that might be used as toxicity criteria in the CLP context. Moreover, lists of 'candidate chemicals' were compiled that would be classified according to the proposed soil toxicity criteria. We come up with a proposal for soil toxicity criteria, which certainly warrants further discussions at EU level in particular in comparison to historic proposals.

4.10.P-Tu233 Comparison of Existing Methodologies Worldwide to Derive Retrospective Ecological Soil Protection Values: A CASE Study With Plant Protection Products

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The Swiss Federal Action Plan on risk reduction and sustainable use of Plant Protection Products (PPP) from 2017 has set the goal of reducing the environmental risk of PPP by 50% by 2027. In this context, it was established that ecotoxicological risk-based reference values (i.e. soil protection values) must be defined for PPP residues in agricultural soils. Although some methodologies for the derivation of soil protection values (SPV) for chemicals have already been proposed under different national or international regulations, most of them have mainly been applied for metals and commonly known organic pollutants. Only few have been used to derive values specifically for PPP. Available methodologies can be very different depending on the country and the regulatory context. In order to assess different performances of the methodologies to derive SPV for PPP residues in agricultural systems, four well-established methodologies used in retrospective soil risk assessment were used in two case studies. The considered PPP were diuron (herbicide) and fluazinam (fungicide). The applied methodologies were from the EU (EC, 2003), Canada (CCME, 2006), Australia (NEPC, 2013) and the United States US EPA (US EPA, 2005). For the same initial dataset, differences along the derivation process led to notable deviations in the final SPV (up to 6 orders of magnitude). One of the major factors responsible were differences in the data selection and in the quality evaluation process of the ecotoxicological data. As a consequence, different data subsets were used depending on the methodology. Also, the SPV extrapolation method (e.g. distribution or assessment factor method) differed between the tested methodologies. Our case studies further highlighted that the effect level considered acceptable can strongly vary depending on the protection goal of the SPV. Most approaches were traditionally developed for contaminated sites and persistent pollutants. Even though some methodologies considered agricultural land use in the derivation process, these ones were rarely applied to PPP (or PPP residues) and when it was the case, specific consideration for this kind of substances in an agricultural context are lacking. Although none of the tested methodologies could fully address the specific goals defined in the AP-PPP, some of them presented the potential to be adapted to consider PPP residues in agricultural fields.

4.10.P-Tu234 Soil Guidance Values: Towards the Long-Term Protection of Soil Quality From Effects of Plant Protection Product Residues in Swiss Agricultural Fields

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Plant Protection Products (PPP) are widely applied in Swiss agriculture and their impact on environmental health is of growing concern. In order to minimize the risk of PPP in the environment and promote their sustainable use, in 2017 the Swiss Federal Council approved an Action Plan for PPP (AP-PPP). PPP can reach the soil either by direct or indirect application and remain in the soil matrix over decades. Therefore, the persistence of PPP residues in the soil and their interaction with non-target soil communities has gained interest over recent years. In order to quantify the extent to which PPP residues affect beneficial soil organisms in in-crop areas, Soil Guidance Values (SGV) should be derived. SGV are intended to be ecotoxicological risk-based reference values (i.e., soil protection values (SPV)) to be used in conjunction with a biomonitoring program. Based on an extensive review of state-of-the-art methodologies for the derivation of SPV, we identified crucial points concerning PPP residues and agricultural land use, which are not covered by the existing guidelines. In this context, and based on the goals of the AP-PPP, the derivation process for SPV needs to be re-evaluated and adapted. For the derivation of SGV under the AP-PPP, data selection should primarily focus on biotests with soil organisms involved in the maintenance of relevant soil functions. Conflicts of objective may arise when the protection of non-target organisms interferes with the effects on the target organisms (e.g. protection of non-target plant species versus pest plant species under herbicide application). Ecotoxicity tests, endpoints and toxicity parameters should be representative of long-term effects (i.e. selection of population relevant endpoints and selection of the effect level). Suitable extrapolation methods have to be identified, especially since the scarcity of soil effect data is a recognized long-standing problem and a general cause of uncertainty for SPV. To this end, considerations and challenges towards a suitable and applicable derivation methodology for SGV are presented, emphasizing the aspects concerning soil quality in agricultural systems and the effect of PPP residues on soil organisms.

4.11 Natural toxins and biopesticides (Poster)

4.11.P-We206 Plant Toxins As WATER Contaminants - Which Are the Controlling Factors?

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Most plants produce secondary metabolites or plant's specialized compounds that are toxic to humans. There are more than 200,000 of such bioactive compounds covering a huge diversity of molecular designs, chemical-physical properties, toxicities and modes of action. Plants produce these "chemical weapons" in their competition with other species, as regulators, and to defend themselves against pathogens or other stressors. Most of the plant toxins (PT) are produced in large quantities in the order of grams per square meter annually. Also, they are relatively polar and hence shows high mobility in the environment. Rain washoff, exudation and plant debris deposition transfer PT from plants to soil, from where they leach to aqueous recipients. The question is if the PTs are sufficiently stable along the track from plant to sink to eventually result in elevated concentrations in water reservoirs - surface water and groundwater - used for drinking water abstraction. Over the past 4 years we have conducted a Marie Curie ITN project with focus on natural toxins (plant toxins and cyanotoxins) and water contamination. The project called NaToxAq (<https://natoxaq.ku.dk>) has resulted in about 50 science papers on the topic. In this contribution we will present learnings and some of the main conclusions for PTs. Many new PTs have been found in natural waters including alkaloids, coumarins, flavanoids and terpenoids at concentrations typically in the ng/L to ug/L scale, and typically with peak concentrations during precipitation events. The fraction of total plant produced PT that leach is often low indicating that PT degradation in plant and in soil is extensive. Alkaloids (nitrogen containing natural compounds) has been identified as a group of prime interest in future studies as these compounds are produced by numerous crop and non-crop plants, some are very toxic and they degrade slowly. A crop-soil-water model (DAISY) has been adapted to describe production, transfer, degradation and leaching of the bracken toxin ptaquiloside.

4.11.P-We207 Comparing the Effects of Tannic Acid and Citronellol on the Physiological Profile of Soil Microbiota

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Citronellol is a terpene with a wide range of chemical properties that suggest its potential to replace commercial products, used as mosquito repellents and/or larvicides. Tannic acid, on the other hand, belonging to the polyphenol family, has a reported antimicrobial activity, effective against different bacteria and fungi, which justifies its use as a preservative in the food industry. Despite such products from natural origin are credited with health safety, their promising application as substitutes for commercial antibiotics calls also for a rigorous assessment of their impact on the environment. This study assesses the ecotoxicity of Citronellol and Tannic Acid on a crop field microbial communities collected from CITA's facilities in Zaragoza, Spain. Both were measured using Biolog EcoPlates®, which contain 31 of the most frequent carbon sources found in crop soils and forests. Their capacity to degrade carbon sources was estimated with the averaged well colour development (AWCD). Five different concentrations of tannic acid (0.2, 2, 20, and 200 and 2000 µg/mL) and of citronellol (0.5, 12.5, 25, 37.5 and 50% in volume) were evaluated and their AWCD values were compared. The results revealed that tannic acid reduced microbial growth even at the lowest concentration in soil environments progressively, being 2 µg/mL the less harmful concentration. By contrast, all assayed concentrations for Citronellol resulted toxic for soil microbiota, affecting microbial community in a similar dramatic way. Without ruling out the possibility of considering them as future pesticides, given their proven antibacterial efficacy, it is also critical to determine their medium-long term impact on ecosystems before making decisions about its commercialization. The authors thank the financial support of Gobierno de Aragón: Departamento de Ciencia, Universidad y Sociedad del Conocimiento (Group E39_20R).

4.11.P-We208 Effects of the Tannic Acid on the Physiological Profile of River Microbiota

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Tannic acid is a polyphenol, found in many plants and their extracts, mainly from their barks and roots. Its emerging utility as an antimicrobial agent and synergist with commercial antibiotics raises the need for an assessment of its long-term toxicity to the environment. This study evaluated the ecotoxicity of tannic acid on freshwater microbial communities from the Gállego river in Zaragoza, Spain. Biolog EcoPlates[®], containing 31 of the most common carbon sources found in forest and crop soils, were used to calculate both the averaged well colour development (AWCD), as an indicator of the entire capacity for degrading carbon sources, and the assortment of carbon sources, as an indicator of the physiological diversity. Four different concentrations of tannic acid (0.2, 2, 20, and 200 µg/mL) were assayed. The results showed a slight increase in AWCD and metabolite consumption for lower concentrations (0.2 and 2 µg/mL), followed by a progressive decrease for higher concentrations (20 and 200 µg/mL). Namely, a decrease in the ability to metabolize, polymers, carbohydrates, and amines/amides was measured. Therefore, this assessment of the metabolic profiles showed that river bacteria communities were affected by tannic acid, suggesting long-term modifications in the structure of river communities. This work allows us to better understand the impact of tannic acid on the environment, a necessary starting point for its future application as an environmentally safe alternative or adjuvant to commercial antibiotics. *The authors thank the financial support of Gobierno de Aragón: Departamento de Ciencia, Universidad y Sociedad del Conocimiento (Group E39_20R).*

4.11.P-We209 Plant Alkaloids in the Soil and Water Environment

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Alkaloids are common compounds produced in plants species of many plant families. Plants appear as single or scarcely distributed in some areas, and as dense populations with high numbers of plants in other areas. The function of the alkaloids are multiple and often associated with regulation or defense against environmental stressors and pests threatening the plant. Competition and evolution create diversity in the chemical structure of the alkaloids, and most plants species present complex mixtures of alkaloids with specialized branches and side groups. The variety of structures of alkaloids present complex mixtures in the plant tissue, and hence the loss and exudation of alkaloids to the soil and water environment is a complex mixture that varies with time. The composition and occurrence varies over time and as response to with environmental stressors. The uncertainty or variation in chemical composition demonstrates the challenges known from the “Unknown or Variable Composition, Complex Reaction products and Biological Materials” (UVCBs) behavior in the environment. Experimental results from plant alkaloid research in field sites studies and lab studies are presented to illustrate current knowledge about occurrence and dynamics for a range of plant alkaloids like pyrrolizidine alkaloids, quinolizidine alkaloids and indole alkaloids. The chemical diversity within these types of alkaloids present in plant tissue over seasons, and exudates to rhizosphere soil and leached to soil solution and nearby freshwater resources is presented. The retention in soil types and transport routes are illustrated with experimental results from the recent Marie Curie ITN project “NaToxAq” Natural Toxins and Drinking Water Quality - From Source to Tap (<https://natoxaq.ku.dk>) with focus on toxins and water contamination. Plant communities of common ragwort, comfrey and butterbur in natural and set-aside areas are studied in connection with streams and rivers. Agricultural crops are represented by sweet lupines as a future valuable protein crop gaining popularity as potential crop adopted to the changing climate, and soybean represent the well-known agricultural crops. Alkaloids impact soil and freshwater and this presentation draw the picture across types and complex mixtures alkaloids in common plant communities.

4.11.P-We210 Ecotoxicological Concerns Associated With Complex and Variable Nature of Botanical Active Substances

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Botanical active substances, or plant extracts/oils, have increased in popularity in recent decades for pest and disease management as alternatives to conventional, synthetic pesticides. According to the SANCO and OECD definition, “a ‘botanical active substance’ consists of one or more components found in plants and obtained by subjecting plants or parts of plants of the same species to a process such as pressing, milling, crushing, distillation and/or extractions. The process may include further concentration, purification and/or blending, provided that the chemical nature of the components is not intentionally modified/alterd by chemical and/or microbial processes.” Whilst this biological origin may lead to a more favourable ecotoxicology profile compared to synthetic chemical active substances, natural occurrence does not imply that the use of such botanical active substances is always without risk and their complex and variable nature can make registration equally challenging. In the EU, botanical active substances fall under the scope of the Plant Protection Products Regulation (EC) No 1107/2009. It is often not technically feasible to radiolabel complex botanical active substances and therefore the SANCO guidance allows one or more component(s) to be identified as analytical “lead component(s)” and traced in the technical grade material. Lead component(s) can be the most frequently occurring substance(s) as demonstrated by analytical techniques or be the most biologically active component(s). However, the identity of the botanical active substance is considered to be the sum of all the components, not just the lead component(s), and this must be considered in the risk assessments. Therefore, it is feasible that other components are present that show a greater ecotoxicological concern compared to the lead component(s). This poster discusses real-life examples and strategies including (Q)SAR, concentration additivity, literature and comparison of other exposures with pesticide exposure to help alleviate ecotoxicological concerns.

4.11.P-We211 Biocontrol of Xylella and Its Vector in Olive Trees for Integrated PEST Management (BIOVEXO)

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Olive cultivation in southern Europe is a long-standing tradition, one that has shaped the environment and the culture in many

European countries. However, there is a growing threat to this part of history. *Xylella fastidiosa* is a pathogen that is increasingly causing diseases on olive trees and various other crops in the Mediterranean region. It wiped out a number of olive groves in Italy and Spain in only a few years, while infections have also been detected in France and Portugal. Unfortunately, the climate of the southern European Union is ideal for *Xylella*, and if the disease continues to spread, it could reduce yields of olive harvests by as much as 70 %. Currently, there are no pesticides available on the market proven to be effective against *Xylella*, which is spread by xylem-feeding insects – notably the spittlebug – common in the Mediterranean climate. Farmers are often forced to destroy infected plants or use chemical insecticides, damaging incomes as well as organic production. BIOVEXO will demonstrate environmentally sustainable and economically viable plant protection solutions that can be deployed as a method of integrated pest management. BIOVEXO's approach involves developing biopesticides that target *Xylella* – X-biopesticides – and those that target the insects spreading the disease – V-biopesticides. The X-biopesticide candidates might be based on an onion extract (a food industry by-product) and antagonistic bacteria; the V-biopesticide candidates might be based on a plant extract (also a food industry by-product), a fungus and a microbial metabolite. Funding: This project has received funding from the Bio-Based Industries Joint Undertaking (BBI-JU) under grant agreement no. 887281. The JU receives support from the European Union's Horizon 2020 research and innovation programme.

4.11.P-We212 Effect of Biopesticide Application on Thiamethoxam Uptake on Maize and Respective Residues on Guttation

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Pesticides are mainly used in agriculture as weed, insect, and fungi control agents and represent important tools in pest management. Low-risk pesticides are proposed as another alternative environmentally friendly, non-chemical approach, which could be included in an integrated pest management schedule. The results of numerous studies showed that the application of biopesticides and arbuscular mycorrhizal fungi (AMF) formulations to crops leads to their symbiosis with the root systems of plants. AMF colonization has many beneficial impacts on crops such as increased nutrient uptake, drought resistance to pathogens, improved root physiology and enhanced plant growth. However, various factors can prohibit AMF to colonize plant roots including high application rates of chemical pesticides and fertilizers, tillage hyphal networks and selective breeding of crop varieties. On the other hand, AMF has also been shown to have unwanted impacts on the plant physiology and composition of beneficial bacterial communities including changes to the chemical composition of root exudates and competition for inorganic nutrients, respectively. The aim of this study was to detect and compare the residue levels of the neonicotinoid insecticide thiamethoxam on guttation when the maize is grown by the use of arbuscular mycorrhizal fungi (AMF). A two-year experiment (2020 & 2021) was conducted under pot conditions. A maize inbred line was selected for both years. The neonicotinoid insecticide Actara® (Syngenta Hellas) was applied alone and in the combination with a commercial formulation containing a mixture of *Trichoderma harzianum* and arbuscular mycorrhizal fungus. In the first application, the insecticide was applied in its recommended dose, while during the second application a double dose was used. The pots were thrice replicated. Guttation was collected in 6 different samplings, in the first morning hours. A HPLC-DAD analysis was used for determining the residue levels of thiamethoxam on guttation. The results revealed that guttation of E treatment of 2020 had residue levels ranged from 0.24 $\mu\text{g/g}$ (1st sampling) to 0.78 $\mu\text{g/g}$ (6th sampling), while guttation of the same treatment of 2021 had residue levels ranging from 0.56 $\mu\text{g/g}$ (1st sampling) to 0.19 $\mu\text{g/g}$ (6th sampling). Results of the 2020 cultivated plants treated with *Trichoderma harzianum* and arbuscular mycorrhizal fungus showed residue levels ranging from 0.26 $\mu\text{g/g}$ (1st sampling) to 1.1 $\mu\text{g/g}$ (6th sampling), while in 2021 of the same treatment showed results ranging from 0.11 $\mu\text{g/g}$ (1st sampling) to 1.66 $\mu\text{g/g}$ (6th sampling). These results suggest that plants that were grown by the combined use of *Trichoderma harzianum* and AMF (treatment M), developed a more dense and extensive rhizosphere that was able to uptake higher quantities of thiamethoxam. The combined application of biopesticides and conventional pesticides may alter the fate and lower doses can be considered in order to prevent the bee colony collapse disorder.

4.12 New developments in the characterization, testing and assessment of complex substances (MCS,UVCBs & MOCS)

4.12.T-01 In Silico Structure Elucidation & FAIR Information Management for Improved UVCB Assessment

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Substances of Unknown or Variable composition, Complex reaction products, or Biological materials (UVCBs) are multi-constituent mixtures that make up approximately a quarter of international chemical registries. Many are high production, import, and usage substances like petroleum, essential oils, surfactants, and solvents. However, despite this high environmental relevance, fundamental information gaps pertaining to substance identity and properties persist. These gaps pose challenges to assessing their hazards, exposure, fate, and potential risks to the environment and human health. In most cases, basic structural and compositional information is not available, which hinders the development of appropriate testing strategies, applying quantitative structure-activity relationship models, fate and exposure modelling, and regulatory decision making. Moreover, what little information that does exist is typically not easily findable, accessible, interoperable, or reusable (FAIR), and is organised using data formats ill-suited to representing these multi-constituent substances with multi-faceted information properties in a machine-readable way. Overall, information availability is inconsistent across both databases and substances within databases (dependent on respective registrants' submissions), and current standard reporting practices/formats of UVCBs' identities do not reflect this phenomenon in a way that supports incorporating uncertainty within UVCB risk assessment. This work to be discussed used cheminformatics

methods to address (i) the specific challenge of missing structure information and (ii) the wider issue of standardising UVCB substance information within databases. First, structure elucidation was achieved by manually searching databases and combining fragment structures while incorporating representative constituent concepts. Assigning structures to UVCB substance names in this way facilitated a real-world regulatory prioritisation exercise. Second, Mixture InChI was explored as a viable cheminformatics format to represent UVCB substances within databases. Various examples of these approaches have been proposed as strategies towards filling critical UVCB identity information gaps and FAIR information management of UVCBs. Together, these initiatives are intended to support risk assessment activities by increasing the availability and accessibility of UVCB substance information.

4.12.T-02 Characterization of Mixtures by Assessing Chemical Similarity

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Many commonly used consumer and industrial products are mixtures composed of multiple chemical constituents. Evaluation of human exposure requires knowledge of a substance's chemical properties; for example, inhalation exposure predictions require input of constituent partial pressures. Partial and total vapour pressures of mixtures may be calculated using Raoult's law, which assumes the various constituents do not interact with each other, *i.e.*, that the system behaves in an ideal manner and the activity coefficient for each component is 1. Depending on the specific mixture components present significant intermolecular interactions may occur; for instance, hydroxyl-containing chemicals may undergo significant hydrogen bonding. These interactions result in deviations from ideality, in which case Raoult's law does not accurately predict vapour pressures and more sophisticated solvation theory methods such as COSMOtherm or UNIFAC may be required. This study aims to assess vapour pressure deviations from ideality expected for different mixture types based on the similarity of mixture components. For a set of binary mixtures, chemical similarity scores were assigned to each solvent pair using 1) Abraham parameters and 2) sigma moments as descriptors. Vapour pressures predicted using Raoult's law and COSMOtherm were assessed using root-mean square error (RMSE) values. A graph of Raoult's law RMSE against similarity scores are shown to give a good correlation for both sets of descriptors, with $R^2 = 0.5579$ for Abraham parameters and $R^2 = 0.5761$ for sigma moments. Results indicate that chemical similarity scores may be a promising metric for assessing deviation from ideality for inhalation exposure.

4.12.T-03 CHANCES2: A Project to Identify Toxicity of a Highly Complex Mixture and to Simulate the Results In Silico

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The Compartmentalised Hazard Assessment for Natural Complex Extracted Substances (type 2) or CHANCES2 project aims to provide greater certainty in the environmental toxicity assessment of Highly Complex Substances for which toxicity is difficult to characterise. The Type 2 Natural Complex Substances (NCS2) UVCB substances of which < 90% of the composition is known (and often << 50%). As well as having the same difficult properties as other NCS (*i.e.* variable composition and volatile and/or hydrophobic constituents), NCS2s contain a large fraction of resins, waxes, *etc.* which makes them particularly difficult to analyse in the laboratory. The aim of this investigation was to propose a new method of hazard assessment for an NCS2 resinoid with a fractionation approach as follows: - Block 1 (the simplest block): corresponds to the fraction of low to intermediate solubility, which is quite volatile. - Block 2: corresponds to the fraction of the more soluble and non- or less volatile constituents, and therefore potentially bioavailable. - Block 3 (the hardest block to characterise): comprising all the other compounds in the NCS2 (insolubles). This fraction is assumed to be inert (or virtually). Further to testing the NCS complete, and then the three fractions separately using acute and chronic testing, an attempt is made to "reconstitute the toxicity" using *in silico* prediction methods adapted for WAF testing as described in Bichere and Thomas (2021). The first step was to analytically separate and then define the fractions. In a second step the fractions (Blocks 1, 2 and 3) were tested using algae, daphnid acute/chronic studies. In parallel, as the analytically defined fractions became available, the WAF toxicities of the two fractions containing identifiable concentrations of constituents were calculated using the iSafeRat® calculator. Next, the results of the toxicity testing and the *in silico* predictions for the first two blocks were compared. Finally, the individual blocks from the test results and *in silico* predictions were collated and the results compared to the acute tests performed on the whole substance. One critical aspect was to ascertain that no chronic toxicity for algae or daphnids could be attained when testing the inert fraction, such that this block could then be subtracted from the hazard prediction of the WAF. The project is ongoing but initial studies have been highly encouraging. Final results are expected to become available in early 2022.

4.12.T-04 Using Weight of Evidence to Assess Degradation Potential of Hydrocarbon UVCB Solvents

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When evaluating the environmental fate of a chemical, it is not uncommon to have a rich dataset from which a conclusion can be drawn. However, the larger the dataset, the more likely there may be conflicting data points. This is due to the inherent variability when working with biological systems, differences in experimental design, and human error. Furthermore, when evaluating degradation potential of a chemical, it must also be recognized that persistence is not an intrinsic property of the test substance itself but may be impacted by a number of factors such as (but not solely): test substance concentration, nutrient availability, source of inoculum and chemical bioavailability. For these reasons when many data points for a single substance exist, it is necessary to evaluate them collectively using a weight of evidence (WoE) approach. WoE is fundamentally a systematic approach

for assessing all data holistically, assigning value to each and making a decision using best professional judgment. Substances identified as Unknown or Variable composition, Complex reaction products or Biological materials (UVCB), in particular hydrocarbon solvents, often have multiple lines of environmental fate evidence that can be utilized when evaluating degradation potential. Hydrocarbon solvents comprise a large group of UVCB substances with carbon number ranges between C5 and C20, and may include normal alkane, isoalkane, cyclic and/or aromatic constituents. The majority are UVCB substances comprising 100's – 1000's of individual constituents. Because hydrocarbon solvents may have a robust dataset including whole substance data as well as data on individual constituents likely to be present in the solvent, a WoE is often necessary to conclude fate properties of a substance. Here we lay out an approach for using WoE to assess degradation potential of hydrocarbon solvents applying four lines of evidence: whole substance testing, data from individual constituents, abiotic data, QSAR models and biological plausibility. These lines of evidence are applied to a case study of a hydrocarbon solvent. Each line of evidence is discussed specific to the solvent, weighed and assessed based on the OCED guidance document for performing weight of evidence. While this approach addresses persistence evaluation, application of this approach can be used for other endpoints for various regulatory frameworks (e.g. REACH).

4.12.T-05 Regulating "More Than One Constituent Substances" Under the REACH Regulation

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1. Introduction Regulating substances with more than one component referred also as “*more than one constituent substances (MOCS)*” (i.e. UVCBs, mono-constituent substances with impurity(ies), and multi-constituent substances with or without impurities) is challenging, as can be associated with (1) the large number of constituents, (2) the composition may be (partially) unknown and/or (3) the composition of the substance may be variable (e.g., seasonal and temporal variation in sourcing of raw materials). Therefore, it is important to, where needed, further develop approaches and guiding principles for the assessment and regulation of these substances. 2. Results and discussion Initial analyses by ECHA revealed that only for ca. 40 % of the UVCB substances registered under REACH a detailed chemical composition could be derived. Recently ECHA has applied some further categorisation of these UVCBs looking at the presence of chemical functional groups within these UVCBs as well as using the available information at composition level. **1. 2. MOCS**, while being substances, are chemically similar to mixtures, so a similar classification approach as for mixtures may need to be considered for MOCS. For the PBT and risk assessments several methodologies are available: *known constituents approach, fraction profiling, whole substance approach or combination of approaches* (e.g. petroleum substances, natural complex substances and inorganic UVCB substances). Community wide regulatory risk management measure has to clearly identify substances falling under the scope of the measure to ensure that (i) industry is able to comply with their obligations, and (ii) authorities can enforce the regulatory measures. The development of further understanding on which regulatory risk management tools are most appropriate to address MOCS is ongoing among authorities. 3. *Table 1 could look like this* Conclusions No unique assessment methodology can be recommended as MOCS can be very diverse. ECHA and industry have developed various guidance documents and tools to facilitate the assessment of those substances. However, there is a need to further develop a common understanding between authorities and industry on the hazard data needed, on how they could be generated and on regulatory risk management actions that can be initiated for MOCS containing constituents of concern. Work is ongoing on those different points in ECHA with the aim to provide more guidance to both industry and authorities on how to handle those complex substances.

4.12 New developments in the characterization, testing and assessment of complex substances (MCS,UVCBs & MOCS) (Poster)

4.12.P-We213 Biodegradation Assessment of UVCBs: Two-Dimensional Gas Chromatography With Peak Tracking of Two Petroleum Substances for Mapping Biodegradation Half-Lives

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Biodegradation is a major removal process for petroleum substances released to the environment, and is controlled by the type of microbial activity and chemical structures present. Most hydrocarbon constituents have the potential to degrade. However, Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs), such as petroleum substances pose a challenge for assigning constituent biodegradation half-lives, since they usually contain thousands of constituents. Only a few hundred constituents are readily quantified in petroleum substances with conventional one-dimensional gas chromatography techniques (e.g. GC–MS), often representing a small fraction of the total substance mass. Two-dimensional GC coupled to a flame ionization detector (GC×GC–FID) can separate and quantify thousands of constituent peaks in petroleum substances. However, matching peaks corresponding to the same constituent(s) on different chromatograms is challenging. We used advanced data-handling algorithms to track the mass changes in constituent(s) peaks across chromatograms corresponding to 7 time points in a laboratory-based biodegradation experiment on two dispersed middle distillate Gas Oils. Several steps were applied: baseline correction; automated peak delineation and peak integration; correction of peak tables for evaporative losses incurred by sample concentration; peak table normalization; peak table alignment; peak tracking; and primary biodegradation half-life calculations. The method was applied to dispersions of the two different Gas Oils that were biodegraded in rotating carousels at 13°C, using

natural non-amended seawater and low oil concentrations (2-3 mg/L). Samples were sacrificed in triplicate over 0–64 d. Hundreds to thousands of peaks were tracked, an order of magnitude more than traditional approaches. We confirmed known biodegradation patterns of oil compounds and extended our understanding to peaks not investigated by traditional methods. Rapid primary biodegradation was observed among the saturated and aromatic hydrocarbons, whereas biomarkers were conserved or show long half-lives. Overall, ~95% of the mass of tracked peaks was degraded during the 64-d experiment. Microbial community analyses showed rapid initial emergence of alkane-degrading bacteria, followed by a community shift after 14 d towards aromatic hydrocarbon degraders and heterotrophic bacteria, the latter associated with utilization of byproducts of oil degradation.

4.12.P-We214 Improving Prediction of Hydrocarbon Biodegradation in Aquatic, Soil, & Sediment Systems Using System Parameterization & Machine Learning

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Evaluation of chemical degradation processes, particularly, biodegradation, is a key element of chemical regulatory management around the globe. Technical complexity and costs associated with environmental biodegradation testing, particularly for UVCB substances, necessitates the advancement of non-testing methods (e.g., quantitative structure-property relationships (QSPRs)). A critical limitation of current models is the inability to incorporate test system and environmental conditions into predictions, leading to uncertainties in model relevance and reliability. This work highlights a novel model (HC-BioSIM) for predicting primary degradation rates (DT50s) of hydrocarbons integrating chemical structure as well as system-dependent parameters, using an expanded database of high-quality petroleum hydrocarbon (HC) DT50 data in water, soil, and sediment systems (N = 728, 1033, & 838, respectively). The HC-BioSIM model (RMSE = 0.34, 0.48, 0.57; R² = 0.52, 0.56, 0.68) significantly outperformed BioHCWin (RMSE = 0.75, 1.2, 0.89; R² = 0.17, 0.13, 0.38) in water, sediment, & soil, respectively. Average errors in predicted DT50s were reduced by 2.5x, 3.9x, & 2.6x. No significant bias as a function of HC class, carbon number, or test system parameters were observed. Finally, k-fold cross validations demonstrated low variability in model performance (R², RMSE) as well as parameter usage/importance. This strongly supports a high degree of generalizability of the model for application to external data. Additionally, the HC-BioSIM models provide improved accuracy of persistence categorization, with correct classification rates of 97%, 90%, & 85% for water, sediment, & soil compartments, respectively. This represents a significant improvement over the existing BioHCWin model (using intermedia extrapolation factors). For the first time, system-specific and environmental effects on the biodegradation of petroleum HCs can be quantitatively evaluated in water, sediment, & soil systems. The model reduces uncertainty and provides a better basis for assessment of environmental degradation for hydrocarbon substances. The model can be applied broadly across regulatory frameworks for risk management. The curation of high quality sediment & soil databases improves understanding of the relative persistence properties of hydrocarbons between environmental media, including assessment of the intermedia extrapolation factors used in persistence assessments and exposure models.

4.12.P-We215 Developing Guidance to Support the Persistence Assessment of Complex Substances - Challenges, Solutions and Case Studies

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Persistence assessment is a cornerstone of chemical hazard and risk assessment and chemical regulation. However, the established frameworks under which chemicals are assessed for persistence and prioritised for further regulatory action have ongoing challenges in their implementation. Complex substances, also known as substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs), are uniquely challenging for persistence assessment. Their compositional complexity and variability can present significant problems for testing and reaching regulatory conclusions. For example, when testing is carried out on whole test substances, concerns have been raised whether the observed biodegradation reflects all constituents of the UVCB, making it difficult to draw a persistence conclusion. Questions also arise as to how the ever-increasing requirements for evaluating mono-constituent substances under existing frameworks should be applied to substances which contain many individual relevant constituents. UVCBs are very broad classes of substances, with widely varying compositional characteristics and complexity. It is evident that there is a need for further guidance to support routine regulatory assessments for complex substances. In this presentation the work of the Cefic-LRI ECO52 project to address these challenges will be presented. Information to support the persistence assessment of UVCBs has been developed to build on that currently documented in regulatory guidance. This has been based on a comprehensive review and consolidation of available information from scientific literature, and the practical experience of the ECO52 project team. UVCBs as a class of substances have been defined in terms of their types and characteristics, with examples. The specific challenges around assessing UVCBs, from both a testing and persistence assessment perspective have been detailed. The presented guidance expands on the ECHA UVCB assessment approach, incorporating novel approaches and developments from the literature, illustrated with case studies. It is envisaged that this information will prove useful to carry out persistence assessments of UVCBs as part of future routine regulatory work. It is clear that persistence assessment of UVCBs remains extremely challenging. Ultimately, bespoke approaches are required that incorporate additional lines of evidence and balance the demands for data with what is practically achievable.

4.12.P-We216 PetroRisk Version 8: Improved Exposure and Risk Assessment for Hydrocarbon UVCBs Using KNIME
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Petroleum substances (PS) contain an indeterminably large and variable number of different constituents, thereby complicating the environmental exposure and risk assessment (ERA) under REACH. ConcaWE's PetroRisk tool facilitates the ERA for such complex substances in line with ECHA Guidance. PetroRisk applies the Hydrocarbon Block Method to extrapolate substance compositional information to concentrations of thousands of representative constituents. For each constituent, the physico-chemical, partitioning and degradation properties, as well as Predicted No Effect Concentrations (PNEC), are embedded in the model in an open database. Subsequently, release estimates, Predicted Environmental Concentrations (PEC), and risks are quantified for each constituent individually. PECs are estimated by multiplying the releases to air, wastewater and soil from each Identified Use (IU) with constituent specific Fate Factors (FF). A use-independent FF database, generated using batch versions of the SimpleTreat and SimpleBox models, is embedded in PetroRisk, thereby avoiding the implementation of these complex multimedia partitioning tools into the model. The resulting constituent PECs are divided by the respective constituent PNECs to obtain Risk Characterization Ratios (RCR). All constituent RCRs are summed up to represent the substance risks resulting from each IU. PetroRisk has now been transcribed into a KNIME workflow. Compared to the previous Microsoft Excel-VBA based PetroRisk versions, the KNIME environment provides a more visual, traceable, and extendable environment, allowing the model to run in batch mode and facilitating the analysis of intermediary data, the communication of results to stakeholders, and debugging. The improved FF concept now provides the user the possibility to refine the exposure assessment. PetroRisk further assists the user in refining the assessment, by ranking emissions according to their contributions to each RCR, and it can implement an optimized set of Risk Management Measure (RMM) removal efficiencies to adequately mitigate undesired risks. Overall, version 8 further simplifies the ERA of complex substances and provides much more support to the user to refine the risk assessments.

4.12.P-We217 Examples of Refined BCF Assessment for Five Surfactants: In Vitro, In Silico, and In Vivo Data
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Surfactants often occur in complex technical mixtures and fall outside the applicability domain of test guidelines and models. A key factor hampering risk assessment efforts for surfactants, is that there are often no, or highly uncertain, octanol-water partition coefficients (Kow). Since many surfactants are produced in high tonnage ranges, information about the bioconcentration factor (BCF) it is required. Most surfactants contain long hydrophobic alkyl chains, and may therefore bioconcentrate above regulatory BCF thresholds. The CEFIC project ECO37 aimed to develop a scientifically sound fish BCF assessment strategy for surfactants that does not rely on Kow. The experimental part of ECO37 included both sorption studies and in vitro assays, as well as in vivo BCF studies, aiming to collect consistent data on systematic series of anionic and cationic surfactants. These data should support the development of mechanistically-based models of surfactant bioconcentration. The membrane-lipid/water distribution ratio (D_{MLW}) is proposed as an experimentally feasible alternative to Kow. For ionic surfactants, phospholipids are the target lipid phase in tissues. In vitro S9 clearance assays with liver extracts from rainbow trout (RT-S9) combined with in vitro-in vivo extrapolation can be used to derive the whole body elimination rate via biotransformation. A tiered BCF assessment scheme for surfactants is proposed, with a baseline partition screening step based on D_{MLW} ($BCF_{baseline}$), and further refinement options to be considered if the $BCF_{baseline}$ is above a regulatory threshold. The current presentation shows the experimental data (in vitro vs. in vivo) and model outcomes for 5 different surfactant examples: 1) a non-biotransformed surfactant (PFOS), for which the screening $BCF_{baseline}$ step is indicative of in vivo BCF; 2) a rapidly biotransformed neutral surfactant (AEO); 3) a rapidly biotransformed anionic surfactant (LAS); 4) pH dependent bioconcentration of an ionizable amine based cationic surfactant (alkyldimethylamine); 5) gill permeation limited BCF for a permanently charged cationic surfactant (alkyltrimethylammonium). These examples display some shortcomings and challenges of the proposed refinement options for BCF assessment and the need to reduce uncertainties in approaches to estimate biotransformation (in vitro/IVIVE, in silico QSAR) to support regulatory acceptance.

4.12.P-We218 A Baseline BCF Screening Approach and Refinement Options for Surfactants
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Surfactants are experimentally challenging chemicals leading to uncertainty in chemical assessment. They often are UVCB mixtures and fall outside the applicability domain of test guidelines and models. A key factor hampering adequate modelling efforts is that there are often no, or highly uncertain, octanol-water partition coefficients (Kow) for surfactants. For some surfactant types, fish bioconcentration factors (BCF) have been measured. BCF values for a variety of nonionic AEO and anionic LAS surfactants are relatively low, due to demonstrated rapid biotransformation. However, BCF is also demonstrated to increase for homologs with more hydrophobic tails. For cationic and zwitterionic surfactants, BCF data are nearly absent. Since many surfactants are produced in high tonnage ranges, and often contain long hydrophobic alkyl chains, reliable model predictions are needed to assess whether the BCF of untested surfactants are potentially above B thresholds, requiring additional (experimental) data. The CEFIC project ECO37 focused on developing a scientifically sound fish BCF assessment strategy for surfactants. The

first aim was to establish a partitioning based BCF screening estimate (BCF_{baseline}) using an experimentally feasible sorbent, equivalent to the Kow approach. Consistent series of membrane-lipid/water distribution ratios (D_{MLW}) are now available for 26 types of surfactants, for a total of ~80 individual surfactant structures. For ionic surfactants sorption to storage lipid (fish-oil) was orders of magnitude lower than D_{MLW} , indicating that phospholipid is the target lipid phase. PFOS is not biotransformed, and hence chosen as a BCF_{baseline} benchmark: a D_{MLW} of 80 000 L/kg and 1.25% phospholipid results in BCF_{baseline} of 1000 L/kg, comparable to reported *in vivo* BCF values of 800-1300 L/kg. The second aim was to evaluate refinement options for the BCF_{baseline} and advanced modeling. Measured *in vivo* BCF for most linear chain surfactants tested on rainbow trout are 1-2 orders of magnitude lower than BCF_{baseline} , confirming a large influence of biotransformation as determined in *in vitro* assays. For permanently charged cationic surfactants (QACs), BCF_{baseline} is overestimated as tissue distribution experiments showed negligible gill permeation. Amine-based surfactants showed pH-dependent BCF, indicating a key role of the minor fraction of neutral species in gill passage, while the D_{MLW} of the ionic form drives internal partitioning.

4.12.P-We219 Environmental Hazard Assessment of Metal Salts of UVCBs

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Environmental hazard assessment criteria are not directly applicable to UVCBs. Here we provide examples for zinc naphthenate, a metal salt containing more than 100 constituents. For biodegradation, QSARs predictions were modelled for representative structures, but metal salts are outside the model domain. Data were read across from naphthenic acids, but regulators identified concerns on reading across from organic components to metal salts due to potential differences in solubility, thus bioavailability, and toxicity. No data were identified for most representative structures and isolation of specific constituents is not feasible. Therefore, biodegradation testing was conducted on the substance itself. The method, adapted from OECD 301, was extended to 60 days and, due to its sticky nature, the test item was mixed with a solid inert carrier before adding to the vessel. It was not feasible to test at a concentration below the NOEC for zinc but results for 100 and 200 mg/L zinc naphthenate showed very little difference in degradation. Ready biodegradability tests are generally not applicable for UVCBs, as degradation may represent removal of only some constituents, therefore it is proposed that all data are combined into a weight of evidence approach, but it is unlikely to be feasible to assess directly against ready biodegradability criteria. Short-term aquatic toxicity tests on zinc naphthenate were conducted as water accommodated fractions and reported as nominal loading rates. Zinc and TOC were measured but specific analysis was not feasible due to the large number and low concentrations of constituents. The presence of zinc is likely to contribute to any toxicity so a T/D test was conducted which gave a 7-day zinc concentration of less than the zinc short-term ERV but a 28-day result above the long-term ERV. A number of approaches have been used to obtain information but it is not clear how this should be interpreted due to a lack of clarity in the current guidance. For metal salts, both the organic and inorganic components need to be considered but, for UVCBs, constituent-based approaches are often not feasible and fail to consider the combined effects and potential differences in bioavailability of constituents. There are limitations with whole substance data and samples should be representative of all potential compositions, which is difficult where compositions cannot be well defined, especially for substances such as zinc naphthenate, which are complex and difficult to characterise, test and analyse. Clear guidance on the assessment of similar substances is required.

4.12.P-We220 Using the RTGill-W1 Cell Line to Investigate Cytotoxic and Molecular Effects of Sediment Extracts From the Agbogbloshie Electronic-Waste Site

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Electronic-waste (E-waste) sites are notoriously contaminated with complex chemical mixtures thus challenging environmental monitoring, management, and remediation activities. Additionally, there is increasing awareness that traditional whole animal based toxicity tests are resource-intensive, expensive, and unethical. Given that E-waste sites are typically situated in low- and middle-income countries that tend to be poorly resourced, there is a need to develop more efficient techniques for application in such settings. The objectives of the current study were to A) perform simultaneous targeted analysis and non-targeted screening of plastic related contaminants in extracts prepared from 35 soil samples collected at the Agbogbloshie E-waste site (Accra, Ghana; classified as upstream-6, downstream-2, community-3, trade site-8, dump site-13, and burn site-3) through liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry (HPLC-QTOF-MS) in full scan mode, and B) to characterize the cytotoxic and molecular effects of these extracts on the rainbow trout (RT) gill cell line RTgill-W1, following the OECD test guideline #249. High concentrations of bisphenol A were measured ranging from 96.80 ng/g (in upstream, downstream, and community) to 255.62 ng/g dry weight (in trade site, dump site, and burn site). Other plastic-related chemicals such as phthalates were also detected, and the concentration of dibutyl phthalate was up to 384.83 ng/g dry weight (in trade site, dump site, and burn site). Gill cells were exposed to concentrations equivalent to 9.38, 4.69, 2.34, 1.17, 0.59, and 0.29 mg dry weight of extract (eQsed)/ml. Many of the samples from the various site types caused a decrease in cell viability % at 9.38 eQsed/ml, for example, two upstream and two community samples (6.9 - 66%), five dump site samples (5.2 - 31.4%), the eight trade site samples (2.2 - 53%), and two burn site samples (59.6 - 77.4%). The trade site group was the most cytotoxic, and the most cytotoxic sample was trade site #8 with cell viability dropping from 74.2% to 2.2% at 0.29 to 9.38 eQsed/ml. Further chemical analysis, and exposures to examine molecular effects of the sediment extracts on the gill cells are underway. This work is expected to support ongoing efforts in establishing the use of efficient alternative testing strategies in ecotoxicology with a focus on developing methods for use in contaminated sites in under-resourced locations.

4.12.P-We221 A Practical Approach to Prioritize Hydrocarbon Solvent UVCB Constituents for PBT Assessment

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Accurate assessment of Persistent, Bioaccumulative and Toxic (PBT) properties of chemical substances is critical for appropriate chemical management decisions. This is difficult for Unknown or Variable composition, Complex reaction products or Biological materials (UVCB) substances, such as hydrocarbon solvents, as existing test methods were designed for single, discrete chemical substances that are often water soluble. This presents challenges as to how best to account for and mitigate inherent uncertainty in evaluating petrochemical fate and hazard data. Hydrocarbon solvents comprise a large group of substances ranging in carbon number between C5 and C20 and containing normal alkane, isoalkane, cyclic and/or aromatic constituents. Here we present a multi-step approach to evaluate representative structures in order to prioritize “worst case” constituents for PBT testing. In this approach, persistence is evaluated first as this is a logical first step to assess PBT/vPvB properties and precludes the need for animal testing. In Step 1, representative structures are selected from the Petrorisk model library (~1500 individual structures) covering the carbon ranges and chemical classes relevant to hydrocarbon solvents. The structures are screened for available peer-reviewed biodegradation data. Structures that demonstrate ready biodegradation are removed from further analysis. In Step 2, remaining structures are assessed through three biodegradation QSAR models. Structures with P/vP results in two or more QSAR models are carried forward for additional analysis; structures identified in less than two QSARS are eliminated from further consideration. Step 3 includes consideration of available bioaccumulation data to eliminate structures with little or no bioaccumulation potential. The remaining structures are those that have been identified in two or more QSARs as P/vP and have either no bioaccumulation data, or bioaccumulation data indicating B/vB. These structures will be tested based on the integrated test strategy (ITS) for persistence, as laid out in the ECHA R11 guidance document. Any constituent where a P/vP conclusion can be drawn at the end of Step 3, will undergo further assessment for bioaccumulation and or toxicity in Step 4 until an ultimate conclusion on PBT/vPvB properties can be reached. We will present the layout, logic and challenges with this approach in identifying “worst case” constituents for PBT assessment.

4.12.P-We222 A Tiered Assessment Framework for Ecological Risk Assessment of Unknown or Variable Composition, Complex Reaction Products, or Biological Materials (UVCBs)

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Complex substances such as multi-constituent substances (MCS) and UVCBs usually result from the industrial processing or extraction of natural substances or from chemical reactions. Because of the nature of source materials, and the potential variability inherent to production processes, these substances can contain many, sometimes uncharacterized constituents, whose concentrations may vary. As a result, UVCB/MCS risk assessment presents unique challenges to product registrants and regulators alike. To address these challenges, the UVCB Committee of the Health and Environmental Sciences Institute is building an exposure-based tiered approach considering the minimum level of information required to perform a robust ecological risk assessment. The first step (Tier 0) considers basic and readily available substance characterization and exposure information. Characterization information includes substance specifications, quality assurance data, and basic chromatographic or elemental data, while exposure information includes use/importation volumes and end uses. Tier 0 information is evaluated to determine whether more characterization information might be needed for a preliminary assessment. This first tier is critical to allow the rapid screening of UVCBs which may or not need in-depth characterization. This framework was tested through the development of three case studies (Cedar Oil, Alkyl Dimethyl Benzyl Ammonium Chlorides, and Hydrogenated Resin Glycerol Ester), which revealed characterization of substance complexity and biodegradation as key steps. Substance complexity, which is likely to determine the needed depth of substance characterization, was defined as the combination of variability in constituent concentrations, diversity of constituent chemistries and chemical properties, and belonging to the applicability domain of existing test models. Also critical is the determination of when biodegradation should be evaluated, and whether it should be measured on the whole substance or representative structures. This approach, which is expected to ensure that efforts and resources deployed for UVCBs risk assessment match actual needs and help streamline the risk assessment process, will be presented and discussed, including the evaluation of substance complexity, biodegradability, and associated challenges. *This abstract does not necessarily represent the views or policies of the U.S. Environmental Protection Agency or CEFAS.*

4.12.P-We223 Use of Tritium Radiolabelled Chlorinated Paraffin Test Substances in Environmental Testing

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The evaluation of chlorinated paraffins (CPs) for environmental endpoints is a priority given recent reviews under the Stockholm Convention and various national chemical regulations. Environmental fate and toxicity testing of CPs is hampered by their very low water solubility, highly lipophilic nature, and compositional complexity, resulting in significant analytical challenges, even with sophisticated equipment and techniques. Tritium (³H) has been occasionally used in environmental fate testing, but not used previously for CPs. An advantage of ³H radiolabelling with CPs is that tritiation is random so radiolabelling occurs at multiple sites along carbon chains as well as in all constituents within the mixture. Also, it results in high specific activity facilitating testing at more realistic environmental concentrations. Moreover, any commercial CP product can be tritiated allowing for direct testing of products on the market. To date, two separate CP substances have been tritiated, n-tetradecane with 30% Cl (wt.) and a

C14-17 commercial substance (MCCP) with 52% Cl (wt.). The first tritiated material was successfully used to confirm and monitor test substance concentrations in a sediment toxicity test (OECD 225). Liquid scintillation counting (LSC) was used to measure the disposition of the radiolabel in the test system and chromatographic techniques were used to determine levels of parent and any metabolites. This same analytical approach and the second material were used to determine the rate and extent of biodegradation of MCCP in activated sludge (OECD 314B). The results showed >90% loss of parent MCCP within 24 hrs and >96% mineralization to tritiated water after 28 days with early formation of transient polar metabolites. These techniques greatly simplified the chemical analysis and allowed for accurate readings and improved mass balances at much lower concentrations not previously attainable. Both of these studies are being used in ongoing evaluations of MCCP in Europe, UK, USA and globally.

4.13 Pharmaceuticals in the environment – New insights, regulatory needs and knowledge gaps (Part I)

4.13.T-01 Feasibility Study of an Active Substance-Based Review System ('Monographs') and Other Potential Alternatives for the Environmental Risk Assessment of Veterinary Medicinal Products

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In the EU, the environmental risk of a Veterinary Medicinal Product (VMP) is assessed for the marketing authorisation in a tiered approach. This product-based ERA system has a number of drawbacks for applicants, competent authorities and other actors. To name only a few, these include test duplication for the same API, the inconsistent evaluation of the environmental risks of similar VMPs containing the same API, the fact that legacy VMPs (authorised before 2005) are not addressed and thus do not have an ERA in line with the guidelines, and the absence of a regular review of the existing risk assessments in view of scientific progress. Both scientific and policy discussions about the shortcomings of the current ERA framework for the authorisation of VMPs and potential alternatives have been ongoing for several years. This has led to the inclusion of Art. 156 in the Regulation (EU) 2019/6 (VMPPR), where a “feasibility study of an active substance based review system (‘monographs’) and other potential alternatives for the environmental risk assessment of veterinary medicinal products” is demanded. An interdisciplinary consortium has been awarded with the study (SANTE/2020/E5/013) which was commissioned by the European Commission’s Directorate General for Health and Food Safety. The study collected information from a systematic literature search and from exploratory interviews with stakeholders to identify sources of relevant evidence and possible data gaps. The data has been used to structure and define the inherent processes of each of the different review systems proposed. This was complemented with information from structured interviews and an online survey with selected stakeholders. The qualitative and quantitative data was analysed to provide answers to the study questions of the call, to evaluate the different review systems for their feasibility and to address inherent uncertainties as well as the advantages and disadvantages to meet the general EU goals. The “monograph system” and two alternatives were examined with regard to their possible impacts as well as their efficiency and effectiveness in achieving the objectives of the VMPPR. The outcome of the feasibility study shall assist the Commission in preparing a report to the European Parliament and Council examining the appropriateness and practicability of moving to a substance-based review system (instead of the current product-oriented review system) and proposing options for further policy action.

4.13.T-02 Advances in Simulating the Fate of Animal Drugs in the US Using VETPEC Modelling Suite

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The United States (US) Food and Drug Administration (FDA) Center for Veterinary Medicine conducts prospective environmental risk assessments to evaluate the potential for significant environmental impacts to occur from the approval of new animal drugs. To date, drug sponsors have relied on third-party tools methods for conducting environmental exposure assessments or calculating predicted environmental concentrations (PECs) of animal drugs. Therefore, US FDA, in collaboration with the US Environmental Protection Agency (US EPA), developed the Veterinary Environmental Transport and Fate Models for Predicting Environmental Concentrations (VETPEC). The VETPEC models calculate PEC values for animal drugs in manure, soil, and water. The modeling suite provides a singular framework that offers the potential to address the regulatory needs described in FDA Guidance for Industry 89 and 166 (Phase I and Phase II Environmental Assessments, respectively), and will provide drug sponsors with an easy-to-use and consistent method to estimate PEC values for use in exposure and risk assessments. In VETPEC v1.0, screening, refined, and mixed-use watershed models have been developed for intensively-reared and pasture-raised livestock. These models consider major environmental fate pathways for animal drug residues including (1) runoff and erosion from manure amended cropland, (2) direct runoff from feed yards and (3) direct runoff from pasture to surface water. The VETPEC models utilize current animal characteristics and US agricultural practices based on US FDA animal scientists’ expert knowledge and information reported in state and producer guidance. As animal drugs approved by US FDA are not restricted to specific locations in the US, the VETPEC models account for differences in soil, weather, and industry practices by leveraging crop scenarios originally developed by the US EPA for model farms throughout the US to predict daily and summarized PEC values for a 50-year simulation. The determination of whether impacts are significant will be based on comparison of these PEC values to predicted no effects concentrations and/or probability of occurrence (i.e., only 1 of the 100 default scenarios may have impacts). VETPEC incorporates tools to aid in the assessment of significant effects, allowing for both the industry investigator and regulatory reviewer to expeditiously evaluate whether effects may occur, how often they may occur, and the proximity of adverse events.

4.13.T-03 Managing Active Pharmaceutical Ingredient (API) Emissions From Manufacturing - an Industry Approach

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Active Pharmaceutical Ingredients (APIs) can be present in the environment, primarily as a result of patient use. Manufacturing of APIs and medicinal products can also result in releases of APIs, however, these have historically been considered to be minor in terms of global load [1]. Whilst there is little empirical evidence to confirm the global contribution of manufacturing discharges in surface waters, at a local level manufacturing discharges could contribute more to the overall load of API and this may be particularly evident on a temporal basis [2]. Importantly, these discharges from manufacturing are within the control of the pharmaceutical industry and as such industry has been working for many years to understand and manage the discharge of APIs to protect the environment. The Pharmaceutical Supply Chain Initiative (PSCI) is a group of pharmaceutical and healthcare companies who share a vision of better social, health, safety and environmental outcomes in the supply chain. As a part of this aim PSCI member companies have prepared case studies demonstrating the management of API discharges. This presentation will share a summary of the approach utilised by industry to assess these discharges along with a small number of these case studies to highlight the work being done at manufacturing sites to improve and reduce the risk posed from API releases from manufacturing sites. Discussion will also summarise potential uncertainties in such assessments and ongoing industry activities to improve assessment and management approaches. References [1] BIO Intelligence Service (2013), Study on the environmental risks of medicinal products, Final Report prepared for Executive Agency for Health and Consumers [2] Anliker, S.; Patrick, M.; Fenner, K.; Singer, H. (2020) Quantification of active ingredient losses from formulating pharmaceutical industries and contribution to wastewater treatment plant emissions, *Environmental Science and Technology*, 54(23), 15046-15056

4.13.T-04 Minimising Experimental Testing on Fish for Legacy Pharmaceuticals

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Various prioritization approaches have been proposed to address the considerable lack of ecotoxicological data needed to assess the environmental risks of legacy active pharmaceutical ingredients (APIs). Minimising, and ideally avoiding, the use of intact vertebrates is an ethical obligation that holds particularly in the absence of a regulatory requirement for ecotoxicological testing as is the case for legacy APIs. Within the research project PREMIER (*Prioritization and Risk Evaluation of Medicines in the Environment*, funded by the Innovative Medicines Initiative 2 Joint Undertaking), ecotoxicological data for approximately 25 case study APIs will be generated. We have developed a decision tree to ensure that *in vivo* testing with fish is avoided for those legacy APIs where other data allow for reliable substitution. A verification dataset of 96 APIs was taken through the decision tree in order to evaluate its robustness (i.e., the number of APIs erroneously excluded from *in vivo* fish testing) and sensitivity (i.e., what proportion of APIs would be considered for *in vivo* fish testing). The proposed decision tree and supporting evidence will be presented and discussed in detail.

4.13.T-05 Recent Developments and Applications of the Exposure to Pharmaceuticals in the Environment, ePiE, Model

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Thousands of active pharmaceutical ingredients (APIs) are detected in surface waters and pose risk to biota and human health. Although substantial monitoring effort is being done in the last decades, measuring all APIs everywhere is not feasible. Therefore, under the correct assumptions, modelling approaches are a solution to determine the APIs' environmental exposure potential everywhere. Oldenkamp et al. (2018) firstly developed the "Exposure to Pharmaceuticals in the Environment" model (ePiE), a numerical model able to predict APIs flow and fate from human consumption to surface waters at 1 km resolution. Within the European project PREMIER (www.imi-premier.eu), we have recently expanded ePiE to cover all the relevant river basins of the European Continent. Furthermore, the model is being enhanced in order to take into account advanced wastewater treatments, which are considered key processes to remove APIs. The new model domain was successfully tested with a hypothetical tracer compound. New applications have shown the model capability of producing enhanced predictions of APIs concentration within Europe. These upgrades together with recent model applications on specific medicines will be presented.

4.13 Pharmaceuticals in the environment – New insights, regulatory needs and knowledge gaps (Part II)

4.13.T-06 Use of Pharmaceutical Sales Data for Improved Prediction of Environmental Risk

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Pharmaceutical production, use and disposal offers a unique set of challenges to scientists and stakeholders, as environmental and health risk must be balanced despite oftentimes conflicting objectives. Despite this, data on the emissions, exposure and effects of pharmaceuticals can be limited or gated behind confidentiality requirements, even in nations at the forefront of environmental regulation. It is imperative that the contributions of pharmaceuticals to environmental risk landscapes in the past, present, and future are better understood, to improve risk characterisation and to ultimately facilitate efficient mitigation. In this presentation, we provide an update on our work applying pharmaceuticals sales data in Norway, 2016-2019, to estimate environmental exposure and thereby help inform on possible environmental risks. We have processed, quality-checked and standardised data on

sales and content of products from the Norwegian Institute for Public Health's Drug Wholesale Database. These data sources are then used to predict environmental concentrations (PECs) for roughly 500 Active Pharmaceutical Ingredients (APIs) per year. For combination drugs, our calculations include all APIs included in each product. Combining these PECs with publicly available Predicted No Effect Concentrations (PNECs), we were then able to calculate Risk Quotients (RQs) for these APIs. Initial notable results are the highlighting of sex hormones, such as levonorgestrel and ethinylestradiol, as key drivers of environmental risk, with RQ values between 100 and 10000. Other notably high-risk substances ($RQ > 1$) include the antiseptic chlorhexidine, the statin simvastatin, the painkillers naproxen, paracetamol, and ibuprofen, the antineoplastic abiraterone and the antibiotics ciprofloxacin and amoxicillin. The current environmental risk assessment procedures typically focus on single pharmaceutical products. Therefore, there is a possibility that the combined effect of drugs with similar modes of action are underestimated. In our study, the total concentrations of APIs from different products will be included in the overall risk characterisation of each API, and will therefore also be accounted for in the risk prioritisation. To our knowledge, our approach provides an unprecedented level of detail and accuracy for predicting environmental concentration, in particular due to our careful inclusion of combination drugs and over-the-counter sales. We intend to next validate our predictions by comparison to measured environmental concentrations in Norway. We also intend to incorporate the calculated exposure and risk into a model for prediction of future environmental risks under changing climate and demographic scenarios.

4.13.T-07 Mixture Risk Assessment of Pharmaceuticals

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There is a growing concern that exposure to these environmental contaminants can result in negative effects on the health of ecosystems due to pharmaceuticals being ubiquitous pollutants, having been detected in surface waters in many regions of the world. Numerous studies have explored the toxicity of pharmaceuticals yet there remain knowledge gaps with only a small proportion of pharmaceuticals in use being tested and these tests primarily being performed on single compounds. The University of York performed a unique monitoring study exploring concentrations of pharmaceuticals in rivers across the globe, providing a foundation for assessing the risks of pharmaceuticals. Therefore, the aim of this study is to use the global monitoring data alongside published ecotoxicity data and predictive models to establish the level of risk posed by individual Active Pharmaceutical Ingredients (APIs) and mixtures of APIs to river systems across the globe. An extensive analysis of current research was conducted to obtain Median Effect Concentrations (EC50) and No Observed Effect Concentrations (NOEC) from chronic and acute apical effect studies and non/apical effect studies of the 53 detected compounds of three taxonomic groups (fish, *Daphnia* and algae). These data were then collated into a database and used to derive predicted no effect concentrations (PNECs) for each compound for surface waters. PNECs and measured concentrations were then used to calculate Risk Quotients (RQ) for pharmaceuticals using a single compound approach (apical and non-apical endpoints and mode of action effects) and a mixture approach at each of the sites monitored. Ten APIs presented an $RQ \geq 1$ in at least one of the 1052 sampling locations (sulfamethoxazole, nicotine, clarithromycin, caffeine, erythromycin, propranol, artemisinin, amitriptyline, and carbamazepine) using apical endpoints. Overall, 12.74% of the sampling locations contained concentrations of at least one pharmaceutical greater than the 'safe' concentrations for aquatic organisms based on apical effect endpoints. Meanwhile, 30.13% of all the sites in the global monitoring had an $RQ \geq 1$ when non-apical endpoints were used, 7.51% when Mode of Action effects were implemented and 24.24% when a mixture approach was applied. Currently, ecotoxicological risk assessments are mainly carried out through apical endpoints; however, this study shows that the use of a single compound approach based on only apical endpoints could underestimate the risk of APIs on non-target organisms in rivers around the globe. Therefore, this work's findings show the importance of utilizing a mixture approach in order to obtain a more comprehensive and 'realistic' perspective of pharmaceutical pollution worldwide.

4.13.T-08 Occurrence, Hazard, and Risk of Psychopharmaceuticals in European Surface Waters

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Psychopharmaceuticals are primarily used to treat psychological disorders and illnesses, as well as other conditions relating to the nervous system and their use has been steadily increasing around the world. After human consumption and excretion, psychopharmaceuticals are emitted into the sewage system and eventually enter surface waters. Since psychopharmaceuticals are designed to interact with the nervous system and alter behaviour, these substances may affect non-target organisms at very low, environmentally relevant, concentrations. Yet, it remains virtually unknown if the presence of these psychopharmaceuticals in surface waters present an ecological risk for aquatic non-target organisms. The current study aimed to provide insights into the risk posed by psychopharmaceuticals and to identify the gaps in the current understanding of these drugs in European surface waters. First, the availability and quality of data on the concentrations of psychopharmaceuticals in surface waters (*exposure*) and on the toxicity to aquatic organisms (*hazard*) were reviewed. Public databases and literature were both used in obtaining these data. If both exposure and toxicity data were available, risk quotients (*RQs*, *risk*) were calculated. In cases where enough toxicity data were available, a species sensitivity distribution (*SSD*) was constructed, from which the hazardous concentration for 5% of the species (*HC₅*) was derived. In total, 703 drugs could be categorised as psychopharmaceuticals based on a combination of all anatomical therapeutic class (*ATC*) 'N' drugs and a list of illicit drugs according to the Dutch Opium Act. Of these, only for 87 compounds enough data were publicly available to calculate RQs. 23 compounds indicated some degree of, with at least one $RQ > 1$. Five of these, i.e. risperidone, escitalopram, carbamazepine, paracetamol, ibuprofen, and fluoxetine had at least 10% of average risk quotients above 1, and therefore carry the highest risk. Yet, only for aspirin, paracetamol, ibuprofen,

trichloroethylene, chloroform, phenol, fluoxetine, sertraline, and carbamazepine risk calculations were reliable in terms of availability and/or quality of data. The results from this study broaden the understanding of the risk of psychopharmaceuticals in the environment by highlighting which compounds are of concern, predicted to be of concern and which compounds lack the data for any calculation of risk.

4.13.T-09 The Emerging Threat of Human-Use Antifungals in the Agro-Environment

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Sustainable farming practices are increasingly necessary to meet the demands of a growing population under constraints imposed by climate change. However, these practices, in particular the reuse of wastewater and amending soil with wastewater derived biosolids, provide a pathway for man-made chemicals such as pharmaceuticals to enter the agricultural environment. A significant knowledge gap remains around the conserved biological potency of human-use antifungals in the environment and, in particular, their potential interaction with plant associated arbuscular mycorrhizal (AM) fungi. AM fungi associations with plant roots play a major role in plant nutrition through their engagement in bidirectional exchange of soil nutrients, in return for plant-fixed carbon compounds. We sought to better understand the impact of human-use antifungals, inadvertently introduced into the soil environment, on the community composition, structure and function of the microbes and fungi that play vital roles in soil nutrient cycling and plant nutrition. Wheat (*Triticum aestivum* L. cv. 'Skyfall') and spring onion (*Allium fistulosum* cv. '(Spring) White Lisbon') and mycorrhizal associates were exposed to environmentally relevant levels of the azole antifungals clotrimazole, miconazole nitrate and fluconazole. Plants were grown under greenhouse conditions as duplicate exposures: Exp. 1 included radiolabelled materials (³³P, ¹⁵N and ¹⁴C) for analysis of fungal colonisation and function and Exp. 2 without radiolabelled materials to track anti-fungal fate and impacts on the diversity of total fungi and the symbiotic AM fungi. We found transfer of essential plant nutrients from mycorrhizal fungi-to-plant was reduced in plants grown in azole-contaminated soils compared to those grown in non-contaminated soils. Interestingly, the observed effects on ³³P transfer from fungus-to-plant occurred without any significant impacts on the degree of mycorrhizal colonisation in response to azole exposure. This suggests that the anti-fungal pharmaceuticals are not at concentrations which are lethal to the fungi or are inhibitory on their ability to colonise plant roots, but they do hamper mycorrhizal facilitation of P transfer from soil to plants. Our work raises the major, yet underexplored, issue of exposure of soil biota to pharmaceuticals such as azole antifungals introduced via sustainable agricultural practices as a potentially globally important disruptive influence on soil nutrient cycles.

4.13.T-10 UTOPIC Database: The Uptake of Pharmaceuticals Into Crops. a Systematic Review and Assessment on the Uptake of Pharmaceuticals Into Crops

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Pharmaceuticals may be released to agricultural environments following irrigation with wastewater effluent or through the application of biosolids or manure. The potential for these pharmaceuticals to be taken up into agricultural crops was first recognised in the late 20th century and pioneering studies on the uptake of pharmaceuticals into crops were conducted in the 2000s. Numerous studies have since investigated the extent of uptake of pharmaceuticals into crops following exposure, but these studies have not been systematically collated or compared. This study aimed to create a database to systematically review all of the existing empirical data on the uptake of pharmaceuticals into crops. This database facilitated a meta-analysis of the data providing an insight into the relationships between the chemical properties of different pharmaceuticals and the uptake pathways in different crops as well as how these vary under different environmental conditions.

Literature searches were conducted on both Scopus and Web of Science with the combined results yielding 1263 discrete articles. These articles were subjected to a robust screening process according to pre-defined screening criteria. The UTOPIC database was designed to systematically store details of the experimental design and results from the 150 studies which satisfied the primary and secondary screening criteria.

The UTOPIC database contains 8048 datapoints spanning 173 separate pharmaceuticals and 78 study crops. Clear trends in experimental design were identified with lettuce being the most studied crop and carbamazepine and sulfamethoxazole being the most studied pharmaceuticals. Significant relationships were identified in the extent of uptake between different crop types. The physicochemical properties of soils were also shown to influence uptake. However, the database highlighted a lack in the reporting of key soil properties limiting the significance of this result. No clear relationships were identified between pharmaceutical chemical properties such as the logKow of the pharmaceutical and the uptake into crops.

The UTOPIC database represents the first aggregation of work on pharmaceutical uptake into crops from scientists across the world. The database provides a wealth of experimental data which could be valuable in the parameterisation of future models. The database is currently being applied to a human health risk assessment and analysis of acceptable daily intake data.

4.13 Pharmaceuticals in the environment - New insights, regulatory needs and knowledge gaps (Virtual Only)

4.13.V-01 Assessing the Potential Impact of Experimental Conditions on Heterogeneous Effect Data for Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) in Aquatic Organisms

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to reduce pain, fever, and inflammation. Due to a steady increase in consumption, aquatic organisms are increasingly exposed to NSAIDs despite generally good removal by WWTPs. Acute adverse effects of NSAIDs are expected to occur at comparatively high concentrations while chronic adverse effects have been reported in the low $\mu\text{g/L}$ range and below. Two prominent NSAIDs, diclofenac and ibuprofen have physicochemical properties that pose a challenge to aquatic toxicity testing. They are weak acids that dissociate at environmentally relevant pH, which changes their solubility, partitioning behaviour, and thus bioavailability. In addition, water solubility is very low. Assessment of aquatic toxicity studies for diclofenac revealed an unexpected heterogeneity of effect concentrations (in some cases covering 3-4 orders of magnitude) within a single species for identical or very similar durations and the same endpoint. In view of the dissociation behaviour and very low solubility, we hypothesize that differences in experimental conditions, e.g. pH, ionic strength, exposure volumes, handling, have resulted in differences in bioavailability of diclofenac. The occurrence of transformation products with dissimilar properties may likewise have affected the results. In order to assess the potential impact of experimental conditions on heterogeneous effect data for diclofenac in aquatic organisms, we are currently curating publically available data on physico-chemical properties of diclofenac to then test the correlation between different experimental parameters and experimental outcomes. A comparison with data already available for ibuprofen will be included.

4.13.V-02 Minimise Harmful Effects of Pharmaceuticals on the Environment: What Are the EU's Objectives?

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The EU legislation on medicinal products is the primary means for ensuring quality, safety and efficacy of medicinal products and their safety for the environment. However, despite the well-established pharmaceutical legislation, risks to and from the environment persist. An analysis of the functioning of the EU pharmaceutical legislation reveals several shortcomings. Improvements are needed especially in the following areas: 1. Strengthening the environmental risk assessment in authorization procedures of medicinal products for human use 2. Closing knowledge gaps in particular for 'legacy' products that were authorised before the requirement of an ERA was established 3. Public access to the results of the environmental risk assessment 4. Improved surveillance in the use phase 5. Effective risk management measures along the entire life cycle of medicinal products 6. Appropriate linkage of pharmaceutical legislation with other regulatory areas. The above mentioned challenges of the EU legislation were also recognised in the EU Strategic Approach to Pharmaceuticals in the Environment, which was published in March 2019. The Pharmaceutical Strategy, published in Nov. 2020 states: "To respond fully to the objectives of a green economy, the regulatory framework needs to address the environmental implications of production, use and disposal of medicines. One of the major challenges is increasing antimicrobial resistance." These objectives are to be implemented in the ongoing revision of the EU pharmaceutical legislation. The Commission's proposal on the new pharmaceutical legislation for human medicinal products is expected in Q4 2022. In addition to the Pharmaceutical Strategy several other EU Green Deal strategies, such as the Zero Pollution Ambition for a toxic-free environment and the Chemical Strategy for Sustainability, which focus e.g. on the revision of the REACH and CLP Regulations and the pursuit of the 'one-substance – one assessment' approach, also contain objectives that will help minimise harmful effects of pharmaceuticals on the environment. A paradigm shift towards a substance-based ERA, the collection of ERA data in a harmonised EU database and a shared use of these data could be the key measures to ensure the environmental safety of medicinal products in use and to enable effective risk management measures along the entire life cycle. A much closer networking of the different regulatory areas is a prerequisite for this.

4.13 Pharmaceuticals in the environment – New insights, regulatory needs and knowledge gaps (Poster)

4.13.P-Tu235 An Integrated Modelling Approach to Assess Water Pollution by Veterinary Pharmaceuticals

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Water pollution by veterinary pharmaceuticals resulting from livestock production is associated with severe environmental and human health risks. While upward trends in global animal product consumption signal that these risks might exacerbate toward the future, veterinary pharmaceutical related water pollution is currently insufficiently understood. To increase this understanding, the presented research assesses processes influencing veterinary pharmaceutical pollution from their administration to their discharge into freshwater bodies, using an integrated modelling approach. For the substances amoxicillin, doxycycline, oxytetracycline, sulfamethazine, and tetracycline we assess loads administered to livestock, excretion, degradation during manure storage, fate in soil and transport to surface water. Fate and transport are modelled using the VA transport model (VANTOM), which is fed with estimates from the Pan-European Soil Erosion Risk Assessment (PESERA). The grey water footprint (GWF) is used to indicate the severity of water pollution in volumetric terms by combining veterinary pharmaceutical loads and predicted no effect concentrations. The approach is applied to the German-Dutch Vecht river catchment, which is characterized by high livestock densities. Results show a veterinary pharmaceutical mass load decrease larger than 99% for all substances under investigation, from their administration to surface water emission. Due to metabolization in the body, degradation during manure storage and degradation in soil, pharmaceutical loads are reduced by 45%, 80% and 90% on average, respectively. While amoxicillin and sulfamethazine dissipate quickly after field application, significant fractions of doxycycline, oxytetracycline and tetracycline accumulate in the soil. The overall Vecht catchment's GWF is estimated at $250,000 \text{ m}^3 \text{ yr}^{-1}$, resulting from

doxycycline (81% and 19% contribution from the German and Dutch catchment part respectively). Uncertainty ranges of several orders of magnitude as well as several remaining limitations underscore the importance to further develop and refine the approach. In this context we specifically discuss the current lack of comprehensive pharmaceutical administration data for livestock. Here we include perspectives on what information at which aggregation level would have significant added value for environmental assessment of pharmaceuticals.

4.13.P-Tu236 Challenges Analysing Monitoring Data for an Insecticide Regulated Under Different Frameworks: A Case Study on Cypermethrin in the UK

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Cypermethrin is a synthetic pyrethroid used as an insecticide in a wide range of products across various sectors, registered under the PPP (1107/2009), biocide (528/2012) and veterinary medicine (2001/82) regulatory frameworks. An important use in the UK is as an ectoparasiticide in veterinary medicines to treat sheep, cattle and horses. Its high toxicity and presence in surface water bodies has led to it being classified as an EC priority substance in 2013. Public water quality monitoring in the UK has identified exceedances of the EQS (annual average and maximum allowable concentration) and this work investigated potential sources of cypermethrin within catchments with a view to identifying sources, exposure pathways to surface water as well as assessing mitigation measures to promote the achievement of good chemicals status compliance. As part of this analysis, potential veterinary medicine sources were assessed using sales data and spatial analysis for a sheep dominated catchment (i.e. animal density and flock treatment specific to the catchment, analysis of managed grass adjacent to surface water, runoff percentage from grassland) alongside three main exposure scenarios (wash-off from treated animals following rainfall events, hardstanding runoff following spillages and drips during treatment and wash-off from animals fording rivers shortly after treatment). Other agricultural sources such as incorrect disposal of products, use of cypermethrin products for fly control in animal housing and losses from livestock sale yards were also considered. Results from the analysis were compared to water quality monitoring data for the sheep dominated catchment and results on the likely sources driving exceedances will be presented. Differences between regulatory environmental risk assessments and on farm practices that can lead to environmental exposure will be discussed as well as the challenges of interpreting water quality monitoring data for chemicals with multiple uses, regulated under different frameworks. The use of different approaches for assessing environmental hazard under veterinary (and other) regulations will also be discussed.

4.13.P-Tu237 Effects of Teflubenzuron on the Early Development of the Sea Urchin *Brissopsis lyrifera* and the Brittlestar *Amphiura filiformis*

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The salmonid aquaculture industry can be affected by biofouling and diseases. Different types of antifouling compounds and veterinary medicinal products (VMP), have been used to control biofouling and parasites such as sea lice. Teflubenzuron (TEF) is one VMP that has historically been used in Norway, but it is very persistent (half-life of 170 days in sediment) and has potentially toxic effects on non-target organisms. The main objective of this study was to assess the effects of TEF on two benthic organisms, representative of the Nordic environment. The sea urchin *Brissopsis lyrifera* was chosen because it can alter sediment grain distribution, while the brittlestar *Amphiura filiformis* is a burrowing organism that uses its arms for suspended feeding. Organisms were collected near the Kristineberg Marine Station (Sweden) in August 2021 using a Van Veen grab (30 m) and a trawl (60 m) for the brittlestar and sea urchin, respectively. Sea urchins were spawned using an intracelomic injection of 0.5 M KCl (ca.1 ml). Subsequently, eggs and sperm were collected for fertilization. Spawning in the brittlestars was induced by photoperiod and gametes were collected for fertilization. Prior to fertilization, sperm mobility, egg quality and gamete compatibility were evaluated for both species. After checking the fertilization success, fertilized eggs (1300 eggs/ml per bottle) were exposed for 5 days to 6 concentrations of TEF (0.001, 0.01, 0.1, 1, 10 and 100 µg/L), alongside a negative (just seawater) and a solvent control (0.01 % methanol). A 5 ml (replicate) sample was extracted daily from each bottle, 2-4 drops of Paraformaldehyde (4%) solution added and the number of eggs/larvae were counted for abnormalities and morphometric analysis using a microscope. On the first 3 days of exposure, length was measured, while on days 4 and 5, body length, body rod length and arm length were recorded. Effects on survival, growth, skeletal development and body symmetry of TEF were analyzed. Data analysis is ongoing but preliminary results point to effects on growth and body symmetry at 100 µg/L for both species. These results are part of the ANTIVENOM project which is assessing the effects of antifoulants and VMPs used in the salmon fishing industry in Norway.

4.13.P-Tu238 Environmental Risk Assessment of Veterinary Medicinal Products - Is a Change From the Current to an Alternative System Feasible and Reasonable From an Operational Perspective?

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Pharmaceutical substances have been detected in surface waters in the European Union for more than 30 years. Since 2005, environmental risk assessments (ERAs) are part of the authorisation process for veterinary medicinal products. The ERA is prepared exclusively based on the product and not on the substance, and thus has a number of drawbacks, which led to discussions about alternative systems. An interdisciplinary consortium was awarded with a contract for a feasibility study of an active substance based review system ('monographs') and other potential alternatives for the environmental risk assessment of veterinary

medicinal products (SANTE/2020/E5/013) which was commissioned by the European Commission's Directorate General for Health and Food Safety. The different systems were examined with regard to their possible impacts as well as their efficiency and effectiveness in achieving the objectives of the VMPP: reduce the administrative burden, enhance the internal market, increase the availability of VMPs, while guaranteeing the highest level of public and animal health and protection of the environment. In this study the following points and questions were addressed to gather important operational aspects to evaluate the feasibility: - For which authorised VMPs are the ERA missing (at the European and national level) - Prioritisation of active pharmaceutical ingredients (API): hazard-based, based on current data gaps, risk-based? - Organisation and governance of a monograph system: responsibility for establishing and updating a monograph, arbitration, data collection, generation of environmental data, data storage, database etc.) - Check for appropriate types of cooperation for applicants – How are experiences from REACH and biocidal product regulation? - How would a possible monograph system fit in the risk assessment system for VMPs? - Challenges while implementing a monograph system - Would alternative systems meet the goals of Regulation 2019/6? - Benefits and challenges for different stakeholder groups (e.g. companies, authorities, the public etc.) The above points were answered by collection of available scientific and grey literature, interviews with stakeholders and an online survey. The collected information for each identified alternative system were structured and analysed for advantages, disadvantages and if they would meet the goals of Regulation 2019/6.

4.13.P-Tu239 Influence of Manure Application Method on Veterinary Medicine Losses to Water

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Veterinary medicines are routinely used within modern animal husbandry, thereof presenting both environmental and societal risks such as antimicrobial resistance and toxicological effects to organisms. The environment is frequently exposed to veterinary medicines via the application of manures to land, however there is currently little understanding regarding the influence of commonly used application methods on veterinary medicine fate. To bridge this knowledge gap, a semi-field study was conducted to assess the influence of manure application methods on veterinary medicine surface runoff and leachate concentrations from both bare soils and pastures. The application methods that were assessed included broadcast, chisel sweep, and incorporation for bare soils whilst the pasture assessment only included broadcast and chisel sweep as incorporation is not representative of typical farming practices. A range of veterinary medicines were selected and applied as a mixture; these were enrofloxacin, florfenicol, lincomycin, meloxicam, oxytetracycline, sulfadiazine, trimethoprim and tylosin. All of the assessed veterinary medicines were detected within surface runoff and leachates, and the concentrations generally decreased throughout the irrigation period. Under the bare soil and pasture assessments concentrations ranged from 0.04 to 309.6 µg/L and 0.33 to 236.83 µg/L, respectively. The total percentage of veterinary medicines that were transported via surface runoff and leachate on bare soils ranged from 0.007 to 0.46%, 0.004 to 0.15% and 0.0003 to 0.11% for broadcast, chisel sweep and immediate incorporation respectively. More advanced manure application technologies (chisel sweep) were observed to reduce surface runoff and leachate concentrations by 13-56% and 49-88% over that of broadcast. Incorporating pig slurries substantially reduced the transport of veterinary medicines, surface runoff and leachate mass loads were reduced by 40-92% and 66-94% respectively. Manure application techniques did not influence surface runoff concentrations under the pasture assessment, although it was evident that chisel sweep can reduce leachate concentrations from 23-97% over that of broadcast. The presented study demonstrates that manure application techniques have a substantial influence on veterinary medicine fate within the environment, and these effects should be considered in the management of manures as a protective risk mitigative measure for aquatic compartments.

4.13.P-Tu240 The Effect of Anaerobic Pig Slurry Redox Potentials on the Degradation of Veterinary Medicines

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Veterinary medicines are frequently used within intensive livestock husbandry and there has been a growing interest regarding their environmental fate following manure application to land. However, research has seldom assessed the influence of pig slurry properties on the fate of veterinary medicines even though such an understanding is essential for a more robust environmental risk assessment. Changes within manure degradation rates have the potential to alter the concentration of antibiotics applied to land, and the outcome of the risk assessment. Research has seldom assessed the influence of anaerobic pig slurry redox potentials on veterinary medicine degradation, this is a precursor of the logistical difficulties that are faced in controlling redox potentials for long durations but here present a pragmatic cost-effect controlling system. The aim of this work was to investigate whether commonly reported redox potentials affect the degradation rates of acetyl-salicylic acid, ceftiofur, florfenicol, oxytetracycline, sulfamethoxazole, and tylosin. The employed redox potentials were -100 mV (reduced), -250 mV (anaerobic) and -400 mV (very anaerobic). A compound specific relationship was observed where the degradation of ceftiofur, florfenicol, oxytetracycline and sulfamethoxazole was inhibited under reduced conditions over that of very anaerobic; the respective DT₅₀ values were 0.7 - 1.84 h, 1.35 - 3.61 h, 22.2 - 49.8 h, 131 - 211 h and 35.4 - 94 h. In contrast, tylosin was found to degrade faster at reduced conditions over very anaerobic (DT₅₀ 6.88 to 19.4 h). The observed changes in degradation rates were found to alter the findings within a risk assessment (i.e., risk quotation to terrestrial and aquatic organisms). Therefore, the presented research demonstrates the importance of redox potential on degradation rates and suggests we need stringent harmonized redox control and numerous manure assessments to improve the environmental risk assessment of veterinary medicines.

4.13.P-Tu241 Monographs for Active Substances in Veterinary Medical Products - Data Gaps and Availability of Environmental Information

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Since 2005 an environmental risk assessment (ERA) has been required for all new veterinary pharmaceuticals within the marketing authorisation procedure. Since then the availability of ERAs is continuously increasing, but there are still veterinary pharmaceutical substances on the market for which no ERA data exists. Art. 156 of the new veterinary regulation (EU) 2019/6 initiated a review of the rules for environmental risk assessment. The recently published “Feasibility study of an active-substance-based review system (‘monographs’) and other potential alternatives” by the European Commission (<https://doi.org/10.2875/94477>) recommends a monograph system. However, information is lacking for how many veterinary pharmaceutical substances complete ERA data are available to draft monographs and for which substances ERA data are still missing. At present, about 500 different veterinary active substances are marketed in Europe. Only a limited number of these are environmentally relevant and used in food producing animals. Following VICH GL 6 and the EMA supporting GL, based on their substance properties or their intended use and the subsequent exposure of the environment, less than 20% would be considered for an in-depth experimental environmental risk assessment according to the VICH GL 38 (Phase II ERA). This poster will list all active substances authorized in Germany for which a Phase II ERA is required. Considering all veterinary medical products marketed in Germany in 2020, we will provide information for which active substances information on their environmental safety is available or still missing. This substance lists will aid in predicting the workload during the implementation of a monograph system.

4.13.P-Tu242 Threats From Pets: Prioritisation of Companion Animal Parasiticides

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Active ingredients within companion animal parasiticide products are of growing concern due to their widespread use and potential high potency to non-target organisms. Current risk assessments do not consider the complete ecotoxicological risk of these chemicals due to the assumed negligible exposure. Selected active ingredients used on companion animals have been detected in the environment. However, environmental occurrence data are available only for a handful of the many active ingredients in use. Therefore, the overall aim of this study was to prioritise active ingredients used in companion animals based on their potential to cause harm to the environment in order to inform future monitoring and risk assessment efforts. Product information on the parasiticides available on the market for treatment of cats and dogs along with ecotoxicological and fate data on the active ingredients used within these products were collated into a custom produced database. Product information collected included the active ingredients used, concentrations of active ingredients and frequency of use. Ecotoxicological and fate data collated included physicochemical properties, persistence, mobility, bioaccumulation and effects on algae, invertebrates and fish. Information has been collated on greater than 100 products; active ingredients from a range of chemical classes are included for example, macrocyclic lactones, neonicotinoids and phenylpyrazoles. These data are currently being used alongside models to identify those active ingredients most likely to cause environmental harm. The future focus of this research is to monitor the high priority active ingredients within the environment to determine baseline concentrations and subsequently to assess the potential risks of these substances to ecosystem health.

4.13.P-Tu243 A One-Health Approach to Control Residual Malaria Transmission - the Value of Ecotoxicology and Environmental Chemistry

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In the fight against malaria, the success of current control tools like insecticide-treated nets and indoor spraying is often undermined by new behaviour of vectors to escape exposure. Vectors may also switch between human and animal hosts. In Burkina Faso, the ANIVERMATE project investigates treating peridomestic cattle with the endectocide ivermectin (IVM) as a complementary malaria vector control tool. Since excreted veterinary drugs can enter the environment and burden ecosystems, ecological consequences of IVM residues are considered upfront. IVM treatments were designed for 6 months and we monitored IVM residues in dung of Zebu cattle, treated with either an innovative injectable long-acting depot or monthly injections of a standard IVM formulation. In addition to IVM residues in fresh dung and blood plasma, we studied the environmental fate of IVM in stored dung and conducted sorption studies with soil samples from local villages. Phytotoxicity was assessed with 2 varieties of *Sorghum bicolor*. Mosquito mortality was evaluated in blood-feeding assays. With an observed 10 d-LC90 of 26 ng/mL in plasma, the depot may reduce residual malaria transmission to close vector loopholes. The long-acting depot resulted in significantly lower IVM peak concentrations in dung (1001 vs 3267 ng/g dw) and excreted IVM showed moderate fluctuations over the trial period. However, ecotoxicological effects of IVM on dung-dwelling Coleoptera and Diptera species are common. For adult dung beetles and flies, IVM in dung may be of lower toxicity but can show severe effects on larvae. Critically, knowledge on local dung and soil fauna is needed: In West Africa, termites and ants play a major role in dung management but risk assessment for these species is not included in guidelines and regulatory frameworks. Integrating ecotoxicological thinking, we show that it is worthwhile to include this mindset early on in human health interventions to reduce adverse impacts on natural resources and agricultural production. ANIVERMATE highlights the importance of including environmental risk assessment in a One-Health approach. The long-acting IVM depot for cattle provided significant mosquitocidal activity for up to 6 months and

may offer complementary and reliable mosquito control with manageable ecological side-effects. However, a One-Health solution can only be truly sustainable if the needs of human and veterinary health, ecology, and ecotoxicology are respected.

4.13.P-Tu244 Environmental Risk Assessment for Small Molecules Used in Targeted Cancer Therapy in Europe

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Environmental Risk Assessments (ERA) according to the EMA ERA Guideline of 1 June 2006 (EMA/CHMP/SWP/447/00 corr 2) were performed for the seven Roche products ALECENSA (INN: Alectinib), COTELLIC (INN: Cobimetinib), ERIVEDGE (INN: Vismodegib), GAVRETO (INN: Pralsetinib), ROZLYTREK (INN: Entrectinib), TARCEVA (INN: Erlotinib) and ZELBORAF (INN: Vemurafenib). These targeted cancer therapy products came on the European market between 2005 and 2021. Due to their high patient safety and efficacy as compared with traditional chemotherapy medicines, these small molecules APIs are increasingly used in cancer treatments. Full ecotoxicity and fate data sets according to the EMA ERA Guideline are available. Wherever possible, the ERAs at the time of marketing authorisation application are supplemented with actual use data from IQVIA MIDAS per annum and country, incorporating population data from Eurostat for the years 2016–2020, in order to evaluate potential environmental risks arising from the effective use of these APIs. Potential environmental risks are not only assessed for surface water, groundwater and sewage treatment, but also for human health via drinking water. Whereas the groundwater assessment is concerned, calculations based on the existing EMA ERA Guideline and the new draft EMA ERA Guideline of 2018 are compared. A potential bioaccumulation is also addressed. Conclusions on potential risks for the aquatic environment of these seven small molecules used in targeted cancer therapy are given in the poster.

4.13.P-Tu245 Environmental Risk Assessment of Medicinal Products - an Investigation of the Generation and Availability of Environmental Data From the Medicinal Product Authorisation Procedure Under European Law

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The pollution of pharmaceuticals in European waters is of concern and risk mitigation measures should further be developed. Environmental risk assessments (ERA) have become part of the authorisation procedure of veterinary/human medicinal products since 2005/06, but they are generated exclusively on a product-by-product basis. This ERA system has several drawbacks, for example the data of the ERA are not sufficient available for authorities responsible for the quality of water in the EU neither for the public. Thus, the overall objective of this legal study was to determine whether and to what extent the generation and availability of data from the ERAs of medicinal products meet the requirements of environmental protection, in particular environmental information law - also in comparison to industrial chemicals, biocides and plant protection products. In the study the following hypotheses were investigated: 1. the medicinal product authorisation procedure is in conflict with the requirements of environmental information law 2. the ERAs of the medicinal products are information on emissions within the meaning of environmental information law and are thus to be published independently of conflicting commercially confidential information 3. the pharmaceutical legislation does not meet the requirements of water protection law 4. the information regimes of the REACH, Biocides and Plant Protection Products Regulation are comparatively effective with regard to the generation and availability of environmental data. In particular, detailed regulations have been implemented on: publication of environmental data, further use of the environmental data and cross-manufacturer use of environmental data. In order to resolve the conflict between pharmaceutical law on the one hand and the requirements of environmental information law and water protection law on the other, the regulations outlined in point 4 should be transferred in a comparable way to pharmaceutical law. In particular, regulations are needed according to which 1. the physicochemical endpoints, information on fate and behaviour in the environment, the results of the ecotoxicological studies as well as the PNEC values are not to be treated confidentially and are to be published, while 2. the study summaries are not to be treated as confidential and published unless the study owner has submitted a reasonable request for confidentiality which has been positively decided by the authority.

4.13.P-Tu246 Human Health Risk Assessment of Pharmaceuticals in the European Vecht River

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Active pharmaceutical ingredients (APIs) can reach surface waters used for drinking water extraction and recreational activities, such as swimming and fishing. The aim of the present study was to systematically assess the lifetime human health risks posed by 15 individual APIs and their mixtures occurring in the German-Dutch transboundary Vecht River. An exposure model was developed and used to assess the combined risks of oral and dermal exposure under a variety of exposure conditions. A total of 4500 API uptake values and 165 lifetime risk values were estimated for 15 and 11 APIs, respectively. Overall, the lifetime human health risks posed by the APIs and their mixtures were deemed acceptable under typical exposure conditions. Under very extreme exposure conditions, API mixture risks were of potential concern while the risks of individual APIs were negligible, with a few exceptions. The antibiotic doxycycline and analgesic phenazone showed the highest and lowest risks, respectively. Recommendations for water managers are provided to help improve the accuracy and utility of human health risk assessments of pharmaceuticals.

4.13.P-Tu247 Human Pharmaceutical Substances - Data Gaps and Possible Solutions

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Since 2006 the assessment of environmental risks for marketing authorisation applications in human pharmaceuticals is based on the guideline on the environmental risk assessment of medicinal products for human use (EMA/CHMP/SWP/4447/00 corr 2), which is currently under revision. For Germany, the German Environment Agency (UBA) is tasked with the evaluation of environmental risks of human pharmaceuticals. For several scientific questions during the revision process of the guideline UBA evaluated the ERA data submitted in the last years and this evaluation is updated now. The general EU human pharmaceutical legislation is also under revision. Strengthening environmental risk assessment is one of the aims of the revision. UBA supports the idea of establishing a catching up procedure for legacy pharmaceuticals connected to a database with publicly available environmental data in substance based monographs. Over the last decade, the regulatory work at UBA resulted in a non-public database containing effect data on approximately 300 active pharmaceutical ingredients (APIs). A considerable part of this data is currently not publicly available due to property rights held by the respective applicants or missing public assessment reports. For our API overview we start with all marketed APIs in Germany. Initially, we identify all APIs for which an ERA is required according to the guideline based on their chemical nature. After comparing them with the database of available ERAs, we focus on German consumption data, pharmaceutical groups with identified environmental risks and also monitoring data from the PHARMS-UBA database (www.uba.de/db-pharm) to identify most relevant APIs without environmental data. The provided API overview is one brick to build up a catching – up procedure for legacy human pharmaceuticals with unknown risks.

4.13.P-Tu248 Pharmaceutical PNECs for Manufacturing Effluents - Lilly's LAEG Program

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Eli Lilly and Company (Lilly) is a Research and Development-focused pharmaceutical manufacturing and sales company. Lilly has a mature manufacturing wastewater management program that ensures compliance with local regulations and protects downstream ecosystems. One part of this program requires adherence to containment limits on the amount of active pharmaceutical ingredients (APIs) that can be discharged to surface water and which are based on Lilly Aquatic Exposure Guidelines (LAEGs). LAEGs are similar to water quality standard (WQS) or predicted no-effect concentration (PNEC) values. These guidelines are set to protect aquatic organisms, humans eating fish and drinking water, and wildlife eating fish and drinking water downstream of Lilly facilities. LAEG values for Lilly APIs are presented along with an overview of methodology for derivation of the values. Lilly facilities are periodically assessed for compliance with containment limits and an overview of those results is presented. The program is being expanded to apply to vendors in the supply chain. An overview of results of compliance for those sites that have been assessed is presented.

4.13.P-Tu249 Prioritization of Active Pharmaceutical Ingredients Based on Predicted Environmental Risk

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The emission of active pharmaceutical ingredients (APIs) to the environment is of increasing concern. Given the enormous number of APIs being used (>1500 APIs in Europe), it is unclear which APIs cause the highest environmental risk and require mitigation. Although an environmental risk assessment (ERA) is mandatory for marketing authorization applications in the European Union, ERA data are not available for most medicines approved prior to 2006. Since it is unfeasible to collect extensive risk assessment data on all APIs, we need to prioritize APIs based on suspected environmental risk and further investigate the top ranking APIs to establish their true environmental risk. Within IMI PREMIER project (<https://imi-premier.eu>), a procedure developed to prioritize APIs based on suspected environmental risk was applied to 1402 APIs used in human medicinal products in Europe. The list of APIs was compiled by combining various inventories, such as EMA's Article 57 database, including medicines authorized in the European Economic Area, the FASS database, including medicines approved in Sweden, and the iPIE?sum database, providing access to various environmental fate and ecotoxicity data of APIs. APIs without a defined and retrievable structure, molecular weight or ATC code were excluded from the list; as were amino-acids, proteins, metal?containing APIs, hormones, antibiotics and molecules with a molecular weight lower than 100 g/mol or exceeding 1300 g/mol. A risk-based prioritization was performed combining an exposure estimate (measured or predicted environmental concentration; MEC or PEC, respectively) with a Predicted No-Effect Concentration (PNEC). We ran multiple prioritizations, using different data sources and proxies, making use of both empirical and predicted data. Starting from easily accessible data, the assessment was then refined using less conservative approaches, requiring more detailed and less accessible data. The number of APIs prioritized decreased as data need increased. We compared the resulting prioritizations to a reference ranking based on empirical data only, and determined the correlation between the relative positions of the common APIs. This correlation decreased as the use of predicted data increased. Ultimately, we identified the top ranking APIs of the prioritizations that better correlate with the reference ranking and we selected 15 APIs with relevant data gaps that will undergo further testing in the IMI PREMIER project.

4.13.P-Tu250 Behavior and Biochemical Effects of Trabectedin in the Zebrafish *Danio rerio*

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Anticancer agents (AAs) pose a great environmental risk due to their increasing consumption and high toxicity to non-target cells. Once used, AAs go to wastewater treatment plants as part of domestic and hospital residues, where they are not efficiently removed. After released into the environment, AAs and their metabolites may promote toxicity (genotoxic, carcinogenic and teratogenic) to non-target organisms due to their modes of action. Among AAs, trabectedin, a cytotoxic AA, used in the treatment

of soft tissue sarcoma, shows no data regarding its environmental risk in the aquatic environment. To start filling in this gap, the aim of this study is to assess the effects of trabectedin at behavioral and biochemical level towards adults of the zebrafish *Danio rerio*. Six fish per replicate (quintuplicates) were exposed to 1.3 nM of trabectedin for 7 days. Fish's horizontal movements were recorded for 12 minutes, alternating 2-minute light and dark periods to provide the total distance travelled by fish and the fish's path angle. Afterwards, fish were returned to their respective aquariums to reduce their eventual stress levels due to handling for one hour, and then they were anesthetized on ice, dissected, and snap frozen for the subsequent enzymes' analysis. Cholinesterase (ChE) activity of the brain and lactate dehydrogenase (LDH) and glutathione-S-transferase (GST) of the muscle were measured through spectrophotometry and were expressed in weight of substrate hydrolyzed per minute per mg of protein. Trabectedin did not affect the total distance covered by adult zebrafish during alternating light and dark periods, as well the path angle rotation. However, statistical differences ($p < 0.05$) between the control and exposed groups were detected in GST and LDH activity, indicating a possible biochemical stress to the fish. The findings presented herein, indicate the increased potential of trabectedin to promote toxicity down to nanomolar range. Concluding, more studies are needed to better understand how trabectedin acts on the zebrafish's metabolic process to provide a suitable hazard prediction of this AA to the environment.

4.13.P-Tu251 Exposure to Environmentally Relevant Concentration of Selected Pharmaceuticals Alters the Life History in the Littoral Cladoceran *Simocephalus punctatus*

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Pharmaceuticals are emerging pollutants widely spread in aquatic ecosystems because its increasing use for human and animal health in the last decades. Despite the presence of these xenobiotics in waterbodies has increased, scarce information about the toxic effects that these pollutants can produce in aquatic biota is available. Medicines are used worldwide, and now concentrations from ng to $\mu\text{g L}^{-1}$ have been reported in many waterbodies, but concentrations as high as mg L^{-1} have also been documented in some rivers. Littoral cladocerans are organisms that can be exposed to water and sludges of wastewater treatment plants containing emergent pollutants that are not removed during treatment. In this study, we assessed the toxic effects of the antibiotic Amoxicillin, the antidepressant Venlafaxine, and the non-steroidal anti-inflammatory Diclofenac. Effects on the life history and growth of *Simocephalus punctatus* were assessed. Neonates of *S. punctatus* were exposed to environmentally relevant concentrations of each drug (0.1, 1, 10, and $100 \mu\text{g L}^{-1}$) for 21 days. Demographic effects were assessed through a Life Table approach determining R_0 , G , e , r , and V_x . The increase in size at 7, 14, and 21 days was also measured. Amoxicillin produced adverse effects on offspring, with 20.35% of the released progeny being dead. Diclofenac exposure increased life expectancy (e) and the net reproductive rate (R_0). Exposure to Venlafaxine decreased life expectancy, but population growth (r) increases in the cladoceran. Generation time (G) decreased in the high concentration of Amoxicillin, but G had no differences in cladocerans exposed to Venlafaxine and Diclofenac. The highest reproductive value (V_x) was observed in organisms exposed to the high concentration ($100 \mu\text{g L}^{-1}$) of each pharmaceutical. At 7-d, the body total length significantly decreased in organisms exposed to the high concentration of Venlafaxine and Diclofenac. At 21-d, organisms exposed to Venlafaxine had the largest body size (2.5 mm). The effects here observed on the reproduction and growth of *S. punctatus* warn about environmental risk caused by the presence of these pharmaceuticals in the aquatic ecosystems. Although not all the observed effects could be classified as unfavorable, other consequences at different study levels (biochemical or physiological) could be expected. Results here presented demonstrated the effects that environmentally relevant concentrations of drugs produced in the aquatic biota.

4.13.P-Tu252 Exposure to the Antibiotic Amoxicillin Produces Toxic Effects in the Cladoceran *Daphnia magna*

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In the past few years, the presence of pharmaceuticals in aquatic ecosystems has received increasing attention due to the adverse effects that these pollutants can produce on the aquatic biota. Among the pharmaceuticals used in many countries worldwide, consumption of antibiotics is in the range of 60 to 70%; within this group, ampicillin, amoxicillin, and bacampicillin are commonly used to treat many diseases in both humans and veterinary medicine. Antibiotics have been recorded in different waterbodies at concentrations ranging from ng/L to $\mu\text{g/L}$. There is little information on the ecotoxicological effects of amoxicillin on aquatic biota, particularly in the zooplankton. For this reason, this study aimed to determine the chronic toxicity produced by amoxicillin in the cladoceran *Daphnia magna*. Acute toxicity was determined, and after this, subchronic toxicity studies were performed. *D. magna* individuals (starting from neonates) were exposed to the sub-lethal amoxicillin concentrations of 0.1, 1, 10 and 100 mg L^{-1} , during 21 d at 25°C, 16:8 photoperiod, and feeding with 8×10^5 cells mL^{-1} of the microalgae *Pseudokirchneriella subcapitata*. Population growth, fecundity, and metabolic biomarkers were measured. Survival, accumulated progeny, and the number of neonates per clutch were quantified daily; the protein, carbohydrate, and lipid concentrations were determined at the end of the bioassay. Results showed that accumulated progeny was significantly higher in the concentrations of 10 and 100 mg L^{-1} of amoxicillin than the control; however, age to first reproduction was significantly lower in 10 and 100 mg L^{-1} of amoxicillin with respect to the control. The number of clutches was not significantly affected at any concentration of the antibiotic. The analysis of metabolic biomarkers in *D. magna* exposed to amoxicillin showed that the proteins, carbohydrates, and lipids increased significantly in the concentrations of 0.1, 1, and $1 \mu\text{g L}^{-1}$ of amoxicillin; nevertheless, biomarkers content decreased at the highest concentration of this antibiotic. Amoxicillin caused significant toxic effects in *D. magna*. Obtained results warn about adverse effects in zooplankton populations exposed to amoxicillin. It is essential to establish permissible limits for antibiotics before they are discharged in aquatic ecosystems, mainly via treated and untreated wastewaters, because of the documented negative effect on the aquatic biota.

4.13.P-Tu253 Feminine Contraception and Environment: A New Estetrol-Based Oral Contraceptive As an Environmentally-Friendly Alternative to Synthetic Estrogens

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Estetrol (E4) is a natural estrogen produced by the fetal liver during human pregnancy. E4 has been recently approved in Europe in a combined oral contraceptive containing 15 mg E4 and 3 mg Drospirenone and is currently under development for its use in hormone replacement therapy. The synthetic estrogen ethinylestradiol (EE2), widely used in oral contraception, is described as a strong endocrine disruptor for aquatic organisms. In order to address the endocrine disruptive potential of E4, a Fish Short-Term Reproduction Assay was conducted according to OECD229 test guideline. Sexually mature zebrafish (*Danio rerio*) were exposed for 21 days to E4 or EE2 at environmentally relevant concentrations. Three concentrations of each hormone were tested: 1, 10 and 100 ng/L for EE2 (0.1 ng/L usually detected in surface waters) and 0.3, 3 and 30 µg/L for E4 (predicted environmental concentration calculated at 0.03 µg/L). Fecundity was quantitatively assessed daily throughout the experiment. At the end of the 21-day exposure period, vitellogenin (VTG) was quantified in body homogenates, and gonads were preserved for endocrine-related histopathology. EE2 considerably reduced egg production at 10 ng/L and significantly stimulated VTG production in both males and females at 10 and 100 ng/L respectively. Histopathology of fish gonads revealed interstitial cell hypertrophy/hyperplasia, interstitial proteinaceous fluid, oocyte atresia, granulomatous inflammation, decreased yolk formation and interstitial fibrosis. Regarding E4, no reduction in egg production was observed from 0.3 to 30 µg/L. However, VTG was significantly stimulated in males at 30 µg/L. Histological analyses of gonads from fish exposed to E4 revealed only a slight interstitial fibrosis in females and a slight interstitial cell hyperplasia and fibrosis in males at 30 µg/L. Estrogens are generally described as endocrine disruptors having strong effects on fish reproduction. However, the results of the present experiment lead to the conclusion that E4 has a much better environmental profile than EE2 and that the use of E4 in oral contraceptives will not affect fish reproduction.

4.13.P-Tu254 Immobile and Defenceless: Behavioural Effects of an Anticonvulsant on *Lymnea stagnalis*

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The majority of human pharmaceuticals are not completely metabolized by the human body, and are thus excreted and subsequently released into the aquatic environment, potentially affecting non-target species. A potentially concerning pharmaceutical is the anticonvulsant topiramate, which reduces neuron firing. Since non-target species could experience similar negative effects as humans, invertebrate behaviour and therewith their role in ecosystem functioning may be affected by the presence of topiramate in the aquatic environment. The aim of the present study was therefore to determine the effect of topiramate on the mobility and anti-predator response of the freshwater snail *L. stagnalis*. The snails were exposed to nominal topiramate test concentrations of 0, 0.01, 0.1, 1 and 10 mg/L, with 5 individually exposed replicate snails per concentration. After 24, 48, 72, 96 and 168 h of exposure, snail orientation was observed for 10 seconds per minute for 10 consecutive minutes and the anti-predator response (retraction and emergence) was scored during 3 minutes after gently probing the snails with a skewer. We observed that the orientation change of the snails exposed to the two highest topiramate test concentrations decreased significantly during the course of the experiment, resulting in a clear dose response relationship for the effect of topiramate on snail orientation, from which an 168 h EC₅₀ value of 0.89 mg/L was derived. The snails exposed to 10 mg/L topiramate also showed a decreased response to probing, with the non-responding snails not retracting into their shell after the stimulus. Hence, a clear dose response relationship was also obtained for the effect of topiramate on anti-predator response, resulting in an 168 h EC₅₀ value of 5.7 mg/L. The negative effects of topiramate on the mobility and anti-predator response of *L. stagnalis* coincide with side-effects in humans, such as psychomotor slowing, ataxia and confusion. Consequently, snails may become more predated and lose their ability to transfer organic material into energy, potentially disturbing the energy dynamics in aquatic ecosystems. Thus, the presence of the anticonvulsant topiramate in the aquatic environment could lead to immobility and defencelessness of invertebrates, potentially affecting ecosystem dynamics. It is therefore concluded that the increased flux of pharmaceuticals into the aquatic environment may alter ecosystem dynamics through changes in invertebrate behaviour.

4.13.P-Tu255 In Vitro Effects of Antibiotics on the Haemocytes of the Clam *Ruditapes philippinarum*

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Pharmaceuticals are biologically active compounds increasingly used in both human and veterinary medicine. Pharmaceuticals are continuously released into the aquatic environments by urban and farm waste waters. This could lead to detrimental effects on non-target species. In this study, we evaluated the *in vitro* effects of three antibiotics: Amoxicillin, Trimethoprim, Ciprofloxacin and – for the first time – a mixture of them on haemocytes of the clam *Ruditapes philippinarum*, a bivalve species widely used in ecotoxicological studies. Haemolymph was collected from the adductor muscle of clams and haemocytes were then exposed for 1 h in culture chambers to filtered sea water (FSW, as control) and to 1 µg/L of individual antibiotics or their mixture in FSW. After the exposure, several cellular responses were evaluated, such as haemocyte viability, lysosomal membrane stability, superoxide anion production, acid and alkaline phosphatase activity, and the frequency of both micronuclei and chromosomal aberrations. One hundred cells per slides (two slides per experimental conditions) were observed under a light or fluorescence microscope at 1000x. Statistical analysis revealed that the haemocytes viability was not affected, but all the tested antibiotics affected the lysosomal membrane stability. In addition, antibiotics and their mixture significantly increased the percentage of cells positive to

superoxide anion. The exposure to antibiotics strongly increased the number of haemocytes positive to acid phosphatase, but not to alkaline phosphatase. Lastly, the exposure did not influence the micronucleus frequency, whereas a significant increase in the frequency of cell aberration (multipolar cells) was observed. These results demonstrated that antibiotics could affect haemocyte parameters in *R. philippinarum*. Furthermore, amoxicillin and mixture showed the highest morpho-functional effects, but the mixture highlighted a non-additional interaction between antibiotics. Although the mechanisms of action of antibiotics on bivalve haemocytes need to be more fully investigated, oxidative stress-mediated toxicity is very likely.

4.13.P-Tu256 Inter-Laboratory and Inter-Exposure-Duration Endpoint Variability of Antibiotics in OECD TG 201 Studies Using the Same Test Organism

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Algal toxicity testing investigates effects on biomass (yield) and the growth rate to multiple generations of the test organisms. Therefore, acclimation and even adaptation may occur during the test period and can accordingly alter test results significantly. Consequently and despite the precise test protocol prescriptions of the OECD Test Guidance 201, which has been updated several times, there is variability in terms of test results from different experiments. This is a generally understood fact and considered in regulation in that the effects on the growth rate, if available, have to be preferred over biomass data. Additionally test ratings are proposed in the literature and used in regulatory practice in order to justify a reliability ranking of the available references. A collection of published data is presented and gives an idea of the order of magnitude of the intrinsic test result variability. The recent OECD TG 201 states a normal test duration of 72 hours. However, shorter or longer test durations may be used provided that all validity criteria can be met. The poster compares example results for antibiotics with test endpoints recorded in the same tests after 72 and 96 h. The influence of different exposure durations is shown and potential reasons are discussed. The order of magnitude of the Inter-Exposure-Duration result variability is compared with Inter-Laboratory variability.

4.13.P-Tu257 It Stays in the Family - Multigenerational Approach for Testing Long-Term Effects of Pharmaceuticals

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Organisms in surface waters are exposed to a variety of pharmaceuticals that were intended for human consumption or agricultural purposes. Because of the (pseudo-)persistence of many of these substances, it seems reasonable to consider an extension of the existing standardized ecotoxicological test methods covering longer exposure scenarios exceeding one generation. Therefore, this study proposes a method for a multigenerational test protocol considering the sensitivity of endpoints (i.e. response variables) and the technical feasibility for a worst case scenario. For this reason, complementary approaches relative to the OECD Guideline 211 were implemented to refine risk predictions and move towards a standardisation of multigenerational testing. For validation, a multigenerational test was performed, in which *Daphnia magna* were exposed to two pseudo-persistent model pharmaceuticals, namely the antibiotic ciprofloxacin and the glucocorticoid dexamethasone. High dexamethasone concentrations (4000 and 10000 µg/L) resulted in 100 % mortality after 7 days, while 640 and 1600 µg/L resulted in a significant decrease in reproduction in the parental generation (F0) after 21 days, which was enhanced in the subsequent generation (F1) under continued exposure. For the endpoint body size, the adverse effects intensified and were most prominent in the F1-generation exposure to 1600 µg dexamethasone/L. The results of ciprofloxacin showed a more complex pattern: body size, weight and reproduction were first significantly stimulated at low concentrations (i.e., 187.5 and 375 µg/L) in the F0-generation, while at high concentrations (i.e., 6000 to 12000 µg/L) opposite effects were found with significantly impaired reproduction, growth and increased mortality. These effects - positive and negative - intensified in the F1-generation. The development of a simplified Dynamic Energy Budget Models could thereby additionally support the scientific understanding on potential stimulatory effects at lower concentrations. Therefore, these additional variables can support the extrapolations to effects on the population level. Hence, the results indicate the potential relevance of a multigenerational test design for the accurate reflection of risks, particularly of persistent or pseudo persistent pharmaceuticals and could further help to refine risk assessment.

4.13.P-Tu258 Opioid Exposure Leads to Differential Behavioural Effects in Zebrafish Larvae

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Opioids find different uses in our society. Therapeutically, they are mainly used to treat acute and chronic pain or serve as a substitution in opioid dependence therapy. Due to their euphoric effect, they are also consumed in a recreational manner. Through human excretion of the parent compounds and metabolites, accidental spillage or disposal opioids are released into wastewater, and are only degraded to a limited extent. Consequently, various opioids can be detected in wastewater influents, effluents and surface water. Since opioid consumption (legal or illicit) is rising, their environmental occurrence is as well. However, their impact on aquatic species is currently only poorly understood. In this study, we used the zebrafish model to investigate the impact of fentanyl citrate and tramadol hydrochloride on the behaviour, physiology and morphology of zebrafish larvae. The light dark transition (LDT) test was used to assess the locomotor activity, which measures the larvae's reaction to alternating light and dark phases. Moreover, larvae were tracked from the side view to assess their vertical positions. Heart rates and swim bladders (SB) were measured from short video sequences recorded from larvae imaged under a binocular microscope. Exposure of zebrafish larvae to fentanyl citrate from 0 to 5 days post fertilization (dpf) lead to a reduced locomotor activity during LDT dark phases and a reduced heart rate at 10 µM. Moreover, the number of animals with uninflated SBs was increased at 10 µM compared to control

conditions, and that the sizes of the inflated SBs were smaller at this concentration. When larvae were exposed to fentanyl citrate and tramadol hydrochloride from 4 to 5 dpf, their SB sizes were expanded compared to control larvae. Moreover, larvae swam mainly close to the surface. SB expansion occurred around 3 hours of exposure to fentanyl citrate, whereas the surfacing behaviour was already induced after about 20 min of exposure. Our results show that different windows of exposure to the opioid fentanyl citrate during zebrafish development lead to differential behavioural, physiological and morphological effects. While developmental exposure leads to sedative effects and SB non-inflation, short exposures lead to uncontrolled surfacing behaviour and SB expansion. These effects can lead to impaired predator avoidance and feeding success and consequently to reduced survival chances of fish early life stages.

4.13.P-Tu259 The Interactions of Antipsychotics With the Cytochrome P450 System of Rainbow Trout and Its Impact on Hepatic Clearance of Pharmaceuticals In Vitro

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The environmental residues of antipsychotic drugs are known to bioaccumulate and cause behavioral changes in fish. We have previously shown that the hepatic clearance of common antipsychotics (haloperidol, levomepromazine, risperidone) in rainbow trout in vitro is deficient or critically dependent on cytochrome P450 activity, which is known to be prone to inhibition by many xenobiotics including these antipsychotics themselves. In the present study, we screened a wider range of antipsychotics (citalopram, mirtazapine, sertraline, venlafaxine and its metabolite O-desmethylvenlafaxine) and anti-inflammatory agents (ibuprofen, ketoprofen, naproxen) for their half-maximal inhibitory concentration (IC₅₀) toward ethoxyresorufin O-deethylation (EROD, CYP1A) and benzyloxy-4-trifluoromethylcoumarin O-debenzylation (BFCOD, CYP3A) in rainbow trout liver S9 fractions (pH 7.8, 11°C). In addition, we determined the impact of P450 metabolism on their overall in vitro clearance based on OECD 319b, by selectively activating (with NADPH) and inactivating (without NADPH) the P450 system in RT-S9 incubations. The in vitro clearance (CL_{int, in vitro}) and IC₅₀ predictions were further corrected by fraction unbound (f_{u,S9}) determined for each compound in RT-S9 with rapid equilibrium dialysis assay. According to our results, all tested anti-inflammatory agents (diclofenac (control), ibuprofen, ketoprofen, naproxen), but only a few antipsychotics (levomepromazine, sertraline), are effectively cleared in RT-S9, and most of them show P450 dependent clearance rates in vitro. Among the test compounds, haloperidol (IC₅₀?10 µM), mirtazapine (IC₅₀?40 µM), levomepromazine (IC₅₀?2.5 µM), and sertraline (IC₅₀?0.3 µM) showed the strongest inhibition toward especially the EROD activity in RT-S9. The relevance of P450 inhibition for the hepatic clearance was further examined through mixture assays with diclofenac and naproxen. As a binary mixture (1 µM each), they did not decrease the hepatic clearances of one another. However, when incubated together with sub-inhibitory concentrations (10 nM each) of haloperidol, levomepromazine, mirtazapine, and sertraline, the clearance rates of diclofenac was decreased by ca. 40% (p < 0,0001, t-test) and naproxen by ca. 30% (p= 0,0002165, t-test). These data indicate the likelihood of and warrant for closer examination of synergistic P450 inhibition in rainbow trout in vitro.

4.13.P-Tu260 The Zebrafish Embryo - a Source of Tools for Risk Assessment of Pharmaceuticals in Marine Ecosystems

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Increasing amounts of therapeutic drugs represent one of the greatest benefits to modern society. However, by their consumption and improper disposal as well as industrial and hospital discharges and wastewater treatment plants, pharmaceuticals are now increasingly being documented in natural ecosystems. First investigated in freshwater systems, they can now also be measured in marine environments. Active pharmaceutical ingredients (APIs) are recognized as important contaminants of emerging concern, but environmental risk assessment can be very complex; in fact, good quality hazard and exposure data are scarce or publicly not available. Ongoing improvement of advanced analytical techniques increased the possibility to detect environmental APIs of different therapeutic classes such as antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), antidepressants and antiepileptics with concentrations ranging from a few ng/L to hundreds of µg/L, but knowledge on fate and biological effects is still fragmentary. Thus, for the development of reliable risk assessment procedures for APIs, basic information such as uptake, bioaccumulation, and excretion kinetics of APIs in aquatic organisms are required in addition to toxicity and teratogenicity analysis, API-specific modes-of-action in non-target organisms and effect evaluations of API mixtures. For that, the zebrafish (*Danio rerio*) represents a versatile tool by providing multiple endpoints for the ecotoxicological assessment of pharmaceuticals such as toxicity studies in early life-stages, the characterization of chronic and subtle effects under ecotoxicologically relevant exposure scenarios, and, thereby, identification of metabolic pathways that play a dominant role in cellular responses to stress. For investigating, e.g., neuroactive drugs, sensory organ toxicity (eyes, olfactory and lateral organs) can be investigated by simple staining procedures and conducting coiling and locomotor assays. Changes in behavior by API can be identified even in embryonic stages. Finally, combining several endpoints of this model system allows to define the most suitable biomarkers for early detection of APIs. Results may be used to adjust prescription and disposal practices of domestic medicines. Supported by BMBF under 724-40003-03F0906A within the PharmaSea consortium (AquaticPollutants Joint Transnational Call by Water JPI).

4.13.P-Tu261 Time and Dose-Dependent Effects of Neuroactive Antidepressants and Pesticides on *Caenorhabditis elegans* Behaviour

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With a rise of antidepressant prescriptions worldwide, there is an increasing concern for the impacts of these pharmaceuticals on nontarget organisms in the environment. Antidepressants are neuroactive compounds and can therefore affect an organism's

behaviour. Behaviour is a sensitive endpoint that can also translate to effects at a population level. Another interesting aspect of antidepressants is that they have shown non-monotonic dose-response (NMDR) curves, with effects at low concentrations that disappear at higher concentrations. Such NMDR relationships may have clear implications for risk assessment, however, the resolution of current studies is often too coarse to have sufficient insights into their relevance. Therefore, the current study was performed into the behavioural effects of the selective serotonin reuptake inhibitors fluoxetine and sertraline and the acetylcholinesterase inhibiting pesticide chlorpyrifos. A wide range of concentrations (ng/l to mg/l) was taken into account and three different types of behaviour were examined: activity, feeding and chemotaxis. In order to statistically examine the non-monotonicity, non-linear regression models (onephasic, biphasic and triphasic) were applied to the results. These models are multiplications of the commonly used Hill model. The results showed a triphasic dose-response curve for activity and chemotaxis after exposure to fluoxetine, but not to sertraline and chlorpyrifos. Effects of fluoxetine already occurred at low concentrations in the range of ng/l. The time dynamics of the effect on activity showed recovery or stabilization over time at higher concentrations. This pattern may be explained by feedback mechanisms, such as inhibition of further serotonin release, the presence of serotonin-absorbing neurons, and receptor desensitization. The effects at low concentrations and the non-monotonicity in the result confirm the relevance of examining such responses, which proves the importance of including a full range of concentrations in order not to miss these effects. Furthermore, this study raises the question of how to include non-monotonicity in mixture toxicity modeling. The nonlinear regression models give multiple EC₅₀s, which is not common in the current mixture toxicity concepts of concentrations addition and independent actions. To assess this, mixture toxicity studies with these compounds are currently being conducted and will be presented.

4.13.P-Tu262 Toxicity Assessment of Tetracycline and Sulfamethazine in *Eisenia fetida* Earthworms

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An increasing global population carried with it the needs of using higher amounts of pesticides and fertilisers in order to satisfy human demands. Antibiotics are widely used to control microbial diseases; especially in livestock maintenance. However, these antibiotics are rapidly excreted and poorly absorbed by target organisms; being released into the environment and spread along with the horse manure. Moreover, slurry and manure are frequently applied in agricultural lands as fertilisers, potentially affecting soil organisms and unbalancing communities. Among the most affected soil organisms, *Eisenia fetida* earthworms must be highlighted due to their close contact with the soil matrix, their representative habitat (horse manure), and sensible response to pollutants. In this work, the toxicity of two different antibiotics: Tetracycline and Sulfamethazine (0, 5, 10, 50, 100, 500, 1000 mg/kg) is evaluated in *E.fetida* earthworms. With such a purpose OECD acute toxicity tests (OECD-204; Filter Paper Contact Toxicity Test and Artificial Soil Tests), OECD reproduction test (OECD-222) and riboflavin measurements in earthworm coeloma were carried out. Thus, digestive or dermal uptake routes are evaluated, allowing obtaining different endpoints at different times and biological complexity levels. After 2 days exposure to sulfamethazine and tetracycline 0, 5, 50, 500, 1000 mg/L concentrations through filter paper, nor mortality nor significant weight losses were noted; highlighting the absence of dermal toxic effects. After 14 days of exposure to sulfamethazine and tetracycline 0, 10, 100 and 1000mg/L concentrations in soil, neither mortality nor significant weight losses were observed among exposed earthworms; discarding acute damages including oral and dermal toxicity routes. However, an increase in Riboflavine content in coelome was recorded in organisms exposed to antibiotics. Earthworms exposed for 28 days to sulfamethazine and tetracycline 0 and 1000 mg/L concentrations in order to evaluate the potential reproductive damages exerted, did not show neither mortalities nor significant weight losses. Similarly, no significant reproductive damages in cocoon production or juvenile number were observed. At tested concentrations no significant effects were recorded at organisms level, however impairments in the immune system of earthworms could occur after exposure to antibiotics. The authors would like to thank: Kontrae project (KK2020-00007) and Basque Government (IT1302-19)

4.13.P-Tu263 Ecotoxicity of Three Widely Used Antibiotics on Non-Target Soil and Water Organisms

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Antibiotics and their metabolites are mainly excreted through urine and faeces reaching aquatic and terrestrial ecosystems through the sewage system. Environment concentrations might produce adverse effects in wildlife and public health due to the emergence of bacterial resistance. The aim of this study was determining the ecotoxicological effects of this type of drugs, namely Chloramphenicol, Tetracycline, and Erythromycin, which are three of the most worldwide prescribed antibiotics. On literature, such compounds were found in environment at concentrations that can reach even 2 µg/L in rivers or 0.51 mg/kg in biosolids and may be subject to bioaccumulation. *Allium cepa* and *Daphnia Magna* were used as biological indicators of terrestrial and aquatic environments, respectively. *A.cepae* was exposed to the following concentrations of the three before mentioned antibiotics: 0.03, 0.3, 3, 30 and 300 mg/L. After a 72-hour incubation, the impact on the root inhibition was measured for the calculation of phytotoxicity on this vascular culture plant and revealed that EC₅₀ values were 102.34 (84.09 - 126.42) mg/L for Chloramphenicol, 106.58 (82.33 - 141.47) mg/L for Tetracycline and > 300 mg/L for Erythromycin. Acute toxicity in *D.magna* was studied using different concentrations for each drug: 200, 400, 600, 800 and 1000 mg/L of Chloramphenicol, 25, 50, 100, 200 and 400 mg/L of Tetracycline and 125, 250, 500, 1000 and 1500 mg/L of Erythromycin. Subsequently, the effects on *D.magna* mobility were measured. The dose-effect curve allowed calculation of the concentration for the test substance whereby a 50 percent of *D.magna* individuals were immobilized after 24- and 48-hours exposition concluding Chloramphenicol EC₅₀(24h) = 285.63 (235.12 - 333.92) mg/L and EC₅₀(48h) = 183.22 (117.31 - 229.32) mg/L; Tetracycline EC₅₀(24h) = >400 mg/L and EC₅₀(48h) = 165.83 (119.17 - 252.53) mg/L; Erythromycin EC₅₀(24h) = 498.72 (405.55 - 608.40) mg/L and EC₅₀(48h) = 197.53

(142.19 - 252.85) mg/L. These results show that antibiotics are not innocuous to non-target soil and water organisms and can produce both acute toxicity in *D.magna* and phytotoxicity in *A.cepa*. Even though EC₅₀ occurs at much higher concentrations than those reported in the environment, the continuous application of biosolids as fertilisers or repeated wastewater discharges do not discard a potential increase in accumulation to higher levels in the coming years. *The authors thank the financial support of Gobierno de Aragón: Departamento de Ciencia, Universidad y Sociedad del Conocimiento (Group E39_20R).*

4.13.P-Tu264 Linking Personality, Gut Microbiome and Response to a Pollutant in Zebrafish

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All animals have unique behavioral patterns termed behavioral syndromes. From an ecotoxicological perspective we know little about how personality affects response to pollutants. Sertraline is one of the the most widely prescribed antidepressants and acts as selective serotonin reuptake inhibitor that increases the concentration of serotonin in the brain. It has frequently been reported as a contaminant in surface waters worldwide. The first aim of this study was to measure the effects of sertraline on the behavior of zebrafish and test the hypothesis that effects will vary depending on personality traits of the individual. The gut biome plays a critical role in regulating host physiology and enhances the development of the central nervous system (CNS) and brain responses. In addition, the gut microbiota interacts with host neuroendocrine system leading to behavior changes. Therefore, the gut microbiome acts as a key intermediary between the external and internal environments. The second aim of this study was to investigate the effects of sertraline on the gut microbiome and relate these changes to personality traits. Zebrafish were exposed to five concentrations of sertraline ranging from 5 ng/L to 5 µg/L for 29 days. All fish were individually tagged and their personality assessed for anxiety and boldness using standard behavioral tests before exposure and again after exposure. Additionally, shoaling behavior and response of the fish to a conspecific alarm substance were tested after exposure. At the end of the experiment, fish were euthanized and the gut microbiome determined using next-generation sequencing. We observed non-linear behavioral responses to sertraline, with fish exposed to 500 ng/L showing a stronger response to the conspecific alarm substance. We also observed treatment-related changes in the gut microbiome. We will discuss how these changes relate to personality responses in the zebrafish.

4.13.P-Tu265 Transcriptomic Profiling of Clobetasol Propionate Induced Immunosuppression in Challenged Zebrafish Embryos

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In the ecotoxicological hazard assessment of chemicals, the detection of immunotoxicity is currently neglected, mainly due to the lack of suitable standardized procedures. However, there are different approaches tackling this issue. For example, immune challenge models using zebrafish embryos can detect significantly reduced mortality rates when the embryos were previously exposed to immunosuppressive substances. Other studies on fish infection models used transcriptomics to get a deeper insight into the underlying molecular mechanisms. In this study, we present a new approach combining the strengths of both methodologies; we performed global gene expression analysis during acute infection with and without potential immunosuppression by chemicals. For this, freshly fertilized zebrafish (*Danio rerio*) embryos were exposed to the immunosuppressive drug clobetasol propionate (CP). After 48 hours, the embryos' immune system was challenged by microinjection of different pathogen associated molecular patterns (PAMPs). Total RNA was extracted three hours post injection and the altered transcriptomic profiles were assessed by RNA-sequencing and bioinformatics analysis. We identified differentially expressed genes (DEGs) regulated by PAMP injection, CP exposure or a combination of both. In order to answer the question whether and how CP impairs an acute immune response, we categorized PAMP regulated DEGs into hyper-, hypo- and non-responsive genes to PAMP after prior exposure to CP. Hypo-responsive genes were of particular interest, because among them there were potential biomarkers for immunosuppression. Interestingly, we observed that the expression rates in the PAMP+CP condition were a result of additive effects from the individual conditions for almost every gene. Overrepresentation analysis was performed in order to get insight into involved biological processes (BP) and we found that the vast majority of enriched ontologies in the PAMP condition were relevant to the immune system. Hypo-responsive genes were predominantly involved in the complement system and in antigen processing, indicating those BP to be most significantly impacted by CP-induced immunosuppression. In this study, we have shown that our approach can be a powerful tool to detect immunotoxicity and identify biomarker candidates. Future validation of this method by testing further model substances could help to increasingly address immunotoxicity in environmental hazard assessment of chemicals.

4.13.P-Tu266 Carbamazepine and Aquatic Macroinvertebrates: Effects on Structure and Functioning and Behaviour

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Current environmental risk assessment of psychotropic drugs fails to reflect on the subtle effects of low drug concentrations. Due to the biologically active nature of psychotropics, their interaction with the nervous system, and their resemblance to infochemicals, these drugs might affect behavior of organisms even at low environmental concentrations. At these low concentrations, effects on the individual survival might seem absent, but small changes in behavior may already result in changes at population and ecosystem level. To account for these subtle effects in the current environmental risk assessment, there is a need for new endpoints to assess effects across different levels of ecological organization (i.e. individual, population, community and

ecosystem). The setup of an outdoor mesocosm study that combines the evaluation of both traditional and behavioral endpoints is presented here. The effects of chronic exposure to 0.001 – 100 µg/L carbamazepine (an anti-epileptic drug) was studied in outdoor freshwater mesocosms for 14 weeks. The main aim of this study is to find correlations between sublethal behavioral effects and changes in the structure and functioning of an aquatic ecosystem as a response to exposure to low environmentally relevant concentrations of carbamazepine. To this end, a set of standard endpoints comprising: abiotic conditions, decomposition of organic matter, Phytoplankton Chlorophyll-a, and the species composition and abundance of zooplankton and macroinvertebrates was measured throughout time. In addition, non-standard behavioral assays investigated the effects on individual feeding behavior and activity of *Gammarus pulex* and *Lymnea stagnalis*. The project will report the effects observed on macroinvertebrate and zooplankton communities as well as on several endpoints describing the effects on individual primary producers and the functioning of the aquatic ecosystem. Determining whether these individual behavioral endpoints are relevant at higher levels of ecological organization could emphasize the relevance of using behavioral endpoints for regulatory purposes.

4.13.P-Tu267 Fate, Dissipation, and Degradation of Psychotropic Medications in Outdoor Mesocosms

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Psychotropic medications, such as antidepressants or anti-anxiety drugs, are some of the most prescribed pharmaceuticals worldwide. They are also some of the most detected pharmaceuticals in freshwaters. With the unprecedented and lasting impacts of the COVID-19 pandemic on people's mental health, prescriptions for these medications are expected to increase globally. The widespread presence of pharmaceutical compounds in the environment has resulted in growing calls for improved risk assessment of this group, requiring high quality data on their fate, behaviour, and effects. Here we provide refined measurements of the fate and half-lives of nine psychotropic pharmaceuticals under semi-field conditions with or without organic sediment. In total, seven outdoor, aboveground, tanks were filled with ~2,300L of fresh water, with a ~15 cm layer of either organic sediment or gravel. Tanks were spiked with a mixture of amitriptyline, bupropion, carbamazepine, citalopram, clozapine, duloxetine, fluoxetine, lamotrigine, and venlafaxine at 50 µg/L (one sediment-containing tank remained un-spiked). Water and sediment samples were collected over 40 days and the concentration of the nine compounds was determined via high-resolution mass spectrometry, which allowed for the assessment of possible degradation products via non-target analysis. For most compounds, the water-column half-lives were less than 2 days, with the most rapid loss in tanks with organic sediment. Only three compounds showed water-column half-lives greater than 2 days, namely Venlafaxine (3d in sediment, 6d in gravel), Lamotrigine (45d sediment, 25d gravel) and Carbamazepine (42d sediment, 30d gravel). Bupropion and Clozapine displayed initial peaks in sediment concentration around 1 week after application followed by a decrease over the remainder of the study. All other compounds showed higher persistence in sediment with concentrations remaining relatively constant over the study duration. A preliminary mass-balance indicates Citalopram, Fluoxetine and, to a lesser extent Venlafaxine, are the most persistent with the total mass of compound (sum of compound in the water and sediment) remaining relatively constant over the 40d study. Common degradation products (e.g. desmethyl-venlafaxine) were also observed and showed similar dissipation patterns as the parent compounds. These data can help us improve existing fate models to better predict their exposure and improve their risk assessment.

4.13.P-Tu268 Psychotropic Drug Interactions in the Freshwater Bivalve *Dreissena polymorpha*: What Have We Learned?

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The potential for pharmaceutical compounds to cause adverse effects on aquatic systems even at low concentrations has become an emerging concern. However, the risks associated with long-term exposures at these low concentrations are still poorly understood, and therefore remain to be demonstrated. Similarly, while drug interactions are well known for humans, studies on cocktail effects on bivalves, in particular, are still rare. World population growth and the increase in the number of people suffering from neuropsychiatric disorders raise questions about the effects of psychotropic drugs, which are frequently found in surface waters, on aquatic organisms. This poster will present some results obtained during the DiMetEP project supported by the French National program EC2CO (Ecosphère Continentale et Côtière), on the interactions between two psychotropic drugs (sertraline and carbamazepine) and a freshwater bivalve *Dreissena polymorpha* commonly used in environmental biomonitoring programs. Through a multi- and interdisciplinary design, taking into account the fate of drugs in bivalves (distribution and metabolism) and their long-term effects, this project provides new knowledge on drug interactions in a representative freshwater species of filter-feeders and empirical data for risk assessment and the development of predictive approaches.

4.13.P-Tu269 Caffeine in Coastal Waters of the Iberian Peninsula: Risks and Possible Use As a Marker of Anthropogenic Contamination

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Caffeine and its main metabolite, paraxanthine, have been widely detected in many environmental matrices, and organisms worldwide. Even though adverse effects at environmentally relevant concentrations have been reported by numerous studies, their

potential environmental risks, and particularly in coastal areas is yet to be determined. Given their widespread consumption, chemical stability, and relationship with other human pollutants, these compounds have been historically suggested as possible markers of anthropogenic contamination. This can be particularly relevant for coastal waters, where current regulations on bathing water quality focus on microbiological endpoints and ignore non-biological pollution. Our study determined the presence of caffeine and paraxanthine in coastal areas of Spain and Portugal, with the aim of testing the usefulness of these compounds as markers of anthropogenic pollution as well as assessing the risk posed by their current levels. Water samples were collected from beach and bathing areas, far from the discharge points of the treatment plants, in 16 sites along the Lisbon and Algarve (Portugal) coast, 18 along the coast of Cádiz (Spain)(spring / summer) and 12 in the Mar Menor coastal lagoon (Murcia). Composite samples were collected every 4 hours for 12 hours. Concentrations of both compounds were detected in all collected samples, despite the fact that all the samples had microbiological levels suitable for bathing. The highest concentrations of caffeine were detected on the beaches of Los Nietos in the Mar Menor coastal lagoon (Murcia), Galapinho/Sierra Arrabida (Portugal), and Chiclana in (Cádiz) (with 704, 315 and 286 ng/L, respectively). The highest values for paraxanthine were also detected in Los Nietos, the Algarve in Portugal and Algeciras in Cádiz (with 216, 205 and 126 ng/L, respectively). Laboratory effects data for chronic exposure was used, when available, for the derivation of Hazard Quotients (HQs). For caffeine, the highest HQ was obtained in Los Nietos (Mar Menor), Chiclana (Cádiz) and Algarve (Portugal) (14.1, 5.7 and 4.9 ng/L respectively). For paraxanthine, the HQ was high (HQ>10) in most sites, with the highest values obtained in Los Nietos, Algarve and Algeciras (54, 51.2, 31.5 ng/L respectively). Based on our results, caffeine and its metabolite can be useful markers of anthropogenic contamination in support of the assessment for water quality in bathing areas. This study has been funded by the Rey Juan Carlos University, through “bridge projects”. Acknowledgements: Ministry of Education and professional training through its program of stays “Senior, Salvador de Madariaga”.

4.13.P-Tu270 Concentrations of Chemicals of Emerging Concern Are Mediated by Seasonal Hydrodynamics in an Offshore Marine Environment

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Many chemicals of emerging concern (CECs), including pharmaceuticals and personal care products, are distinct from legacy contaminants in their ability to travel readily in aqueous matrices, resulting in the common occurrence of CECs in aquatic systems receiving point and nonpoint source waste streams. However, little data currently describes CEC concentrations in marine ecosystems. Here we measured 17 pharmaceuticals, caffeine, sucralose, and 25 per- and polyfluoroalkyl substances (PFAS) surrounding a major wastewater discharge into Massachusetts Bay, USA. Water grabs were collected from surface and bottom water compartments, and field work was conducted during both stratified and unstratified water column conditions. Samples were analyzed for CECs using liquid chromatography coupled to tandem mass spectrometry. 10 of 17 pharmaceuticals, sucralose, caffeine, and 9 PFAS were detected in surface and bottom water at low (>40 ng/L) to very low concentrations (>2ng/L). Concentrations were proportional to proximity to the point source discharge, with the highest concentrations observed directly south of the outfall in accordance with regional circulation patterns. Stratified conditions mediated occurrence of CECs, with increased bottom water CEC concentrations during stratified conditions and increased surface water CEC concentrations during unstratified conditions. Concentrations of all analytes in both surface and bottom water were generally higher in winter, likely related to reduced biological degradation and uptake. This work highlights the potential for pharmaceuticals and other CECs to persist in marine environments, and underscores the need for further evaluation of potential impacts of these compounds in marine biota subject to chronic, low-level exposures.

4.13.P-Tu271 Environmental Fate of Estetrol (E4), a Native Estrogen Used in Oral Contraception

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Estetrol (E4) is a unique native estrogen produced by the human fetus during pregnancy. E4 formulated as the monohydrate is a new active substance that received a positive opinion from the Committee for Medicinal Products for Human Use (CHMP) for use in a combined oral contraceptive. E4 is projected to enter the aquatic environment through effluents discharged by sewage treatment plants. The environmental fate of E4 was investigated using a battery of studies designed to describe its physical/chemical characteristics and potential for persistence and bioaccumulation in the environment. The mean measured octanol/water partition coefficient (log KOW) of E4 was determined to be 1.65. In a ready biodegradability study, the mean biodegradation was 3% after 28 days incubation. E4 is not significantly sorbed to soils and sewage treatment solids with Freundlich isotherm adsorption coefficients Kf between 88 and 147 in soils and 35 and 36 in sludges, respectively. In a 100-d aerobic-aquatic biodegradation study into two systems, E4 biodegradation rates in water were 0.182/d and 0.057/d (half-life 3.81 and 12.08 d, respectively) and in sediment were 0.103/d and 0.169/d (half-life 6.71 and 4.09 d, respectively). The results presented illustrate the transient nature of E4 in the aquatic environment and indicate that bioaccumulation of E4 would not be expected.

4.13.P-Tu272 Emissions of Imidacloprid From Sewage Treatment Plants From Use of Veterinary Medicinal Products in the UK

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Imidacloprid has been detected in some water samples taken from European rivers. Potential sources of imidacloprid include historical uses in outdoor and greenhouse plant protection products (ending end May 2022), in imported potted plants, fruits and vegetables, as well as in biocides and veterinary medicinal products (VMPs). In a previous work, we examined VMPs as a potential emission source of imidacloprid through modelling work considering the following 3 exposure pathways: 1. washing of dogs, 2. washing of pet bedding of cats and dogs and 3. walking dogs in the rain. The conclusion reached was that Imidacloprid containing VMPs make only a very small contribution to the levels of Imidacloprid detected in UK surface waters. The calculated environmental concentrations indicated an acceptable risk to aquatic organisms. Two additional exposure scenarios have been developed considering Imidacloprid containing spot-on and collar VMPs: 1. abrasion from treated animals to people's clothes and washing of these clothing followed by release to wastewater to the Sewage Treatment Plant (STP), and 2. release after stroking of cats and dogs followed by hand washing and release to surface water via STP. General parameters based on the Biocidal Product Regulation (BPR) or on product specific studies as used in the first three scenarios were taken into consideration for the new emission pathways with addition of scenario specific parameters. The aim of this further work was to model the additional pathways and to compare the Predicted Environmental Concentrations (PEC) of each scenario against the Predicted No-Effect Concentration (PNEC) of 4.8 ng/L (BPR). Additionally, the PEC values were compared against the draft 2021 Annual Average Quality Standard (AA-EQS) of 6.8 ng/L. This value was recently proposed by SCHEER in context of an update of the watchlist of the EU Water Framework Directive. Imidacloprid surface water concentrations calculated for the three original scenarios were also compared with the same chronic aquatic ecotoxicological endpoint. The calculated surface water concentrations for the five scenarios, which consider worst case situations, do not exceed the ecotoxicological thresholds indicating acceptable risks for aquatic invertebrate organisms. The models developed demonstrate a low exposure to the aquatic environment from the use of Imidacloprid containing VMPs.

4.13.P-Tu273 Pharmaceutical Bioavailability Regulation by pH and Dissolved Organic Carbon: A Common Mechanism?

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Since last century, adverse effects of anthropogenic chemicals on aquatic organisms have been identified and lead to progress in environment protection regulations. Yet, the basis of regulations is often risk assessment conducted in controlled laboratory conditions, but the toxicity of chemicals in natural environments are regulated by dynamic water chemistry factors like pH and dissolved organic carbon (DOC). At the SETAC Europe 31st annual meeting, we have presented a bioavailability model for the antibiotic ciprofloxacin (CIP) with the possibility to predict CIP toxicity under dynamic environmental conditions. Here, we will report on the impact of pH and DOC on the toxicity of ionizable pharmaceuticals with varying octanol water partition coefficient (K_{ow}) and acid dissociation constants (pK_a). Experiment results were applied to parameterize the key section of our model: an equilibrium module consisting of pH-dependent chemical speciation and DOC related binding processes, in order to examine how well the bioavailability model might be extrapolated to ionisable chemicals with properties distinct from CIP.

4.13.P-Tu274 Sustainable Treatment of Wastewaters Generated at a Chemotherapy Unit Through the Sequential Use of Microbial Fuel Cells and Constructed Wetlands

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Due to a higher prevalence of cancer cases, there is currently a considerable increase in relation to the consumption of drugs used in chemotherapy treatments. These anticancer drugs (also called cytostatic) have been frequently detected in several aqueous matrices and because of their non-selective modes of action, that is, attacking both cancerous and healthy cells, they have a high toxic potential. Despite the low concentrations found in aquatic environments, when compared to other classes of drugs, several authors have repeatedly stated that, due to their cytotoxic, genotoxic, mutagenic and teratogenic potential, it is possible that these compounds may cause several damages to aquatic biota even at very low concentrations. In addition, such compounds may also have their toxicity magnified due to possible synergistic effects. Finally, it should be noted that cytostatic drugs have a refractory nature and thus are not removed/eliminated by conventional wastewater treatment systems. In this sense, the present study aims, through the sequential use of microbial fuel cells and constructed wetlands, to propose a sustainable alternative, both from an environmental and economic point of views, for the treatment of wastewaters generated at a hospital chemotherapy unit. The integrated system will be composed of a microbial fuel cell unit planted with *Chrysopogon zizanioides* and compartmented with glass wool barrier in order to control cytostatic residues and eventual degradation products, frequently detected in the effluents generated at the oncology center of the Ana Nery Hospital, and a constructed wetland unit vegetated with *Hymenachne grumosa*. The study will focus its actions on the degradation/elimination of these anticancer compounds, focusing mainly on toxicity tests since many of these cytostatic are not mineralized but only partially eliminated, generating by-products that can be as or even more toxic than the parent compounds and on the generation of bioelectricity. Thus, in addition to reducing the negative impacts resulting from the inadequate release of these effluents, the bioelectricity generated from the microbial cell may eventually be used in combinations of advanced oxidative processes that are integrated into the system, such as electrooxidation, UV radiation and ozonation.

4.13.P-Tu275 The Role of Seasonal Water Temperature Changes in Bioconcentration and Biotransformation of Psychoactive Compounds and Their Mixture in Wild Fish

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Pharmaceutically active compounds (PhACs) have been shown to accumulate in aquatic and riparian food-webs. Yet, our understanding of how temperature, a key environmental factor in nature, affects uptake, biotransformation, and the subsequent accumulation of PhACs in aquatic organisms is limited. In this study, we tested to what extent bioconcentration of three psychoactive pharmaceuticals is affected by two temperature regimes (7 and 20°C) and how the temperature affects the biotransformation of parent compounds and subsequent accumulation of their main metabolites in experimental organisms. Selected pharmaceuticals represent a tricyclic antidepressant Amitriptyline, a selective serotonin reuptake inhibitor Sertraline and serotonin–norepinephrine reuptake inhibitor Venlafaxin. As an experimental organism, we used wild European perch (*Perca fluviatilis*) which represents both predator and prey species of high ecological relevance in food chains of boreal and temperate aquatic ecosystems. Fish were exposed to individual drugs as well as their mixture at concentration of 1 µg L⁻¹ and two temperature regimes resulting in eight different treatments. To describe the pharmacokinetics of target drugs, five individuals were sampled at 10 sampling points during the 14 days of exposure period within each treatment. Samples of blood plasma and brain were obtained from each sampled individual and are being analyzed using the liquid chromatography tandem mass spectrometry (LC-MS/MS) instrumentation. Parent compounds and their main metabolites will be quantified in each sample to describe the role of the temperature in pharmacokinetics of these antidepressants and possible differences in bioconcentration scenarios between warm and cold seasons of the year. Given that temperature is a fundamentally important environmental variable for ectothermic aquatic organisms, and is known to influence standard metabolic rate in fish, we expect that it would have significant effect on uptake and especially biotransformation of PhACs in fish. Such data are missing at the moment, but would be beneficiary for improving the models that start to be used for environmental risk assessment of pharmaceuticals (e.g. fish plasma model). As these models are extrapolating data from mammalian pharmacology, which are based on a stable body temperature, important variable might be missing to predict effects in aquatic ectotherms.

4.13.P-Tu276 Method Development for the Assessment of Bioaccumulation Potential of Pharmaceuticals in Fish

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Active pharmaceutical ingredients (API) represent a group of the most prominent categories of emerging environmental contaminants. However, a vast majority of the currently published studies are focused on their occurrence and fate in abiotic environmental matrices like water and sediments while the data on API concentrations in aquatic organisms are still comparatively rare. Consequently, the existing knowledge on API bioaccumulation and metabolic transformations in the aquatic organisms is still inadequate. The key prerequisite to study bioaccumulation behavior of pharmaceuticals in the aquatic environment is the availability of analytical methods suitable for the reliable determination of trace concentrations of API and their metabolites in complex biological matrices. In this work, a novel multiresidue LC-MS/MS method for quantitative measurement of trace levels of selected APIs and their metabolites in freshwater fish was developed. In order to develop a robust analytical protocol suitable for reliable determination of 44 analytes, characterized by rather wide-range of physicochemical characteristics, in difficult fish matrices, the sample preparation protocol was optimized in a number of model experiments, by examining different combinations of extraction and clean-up techniques. The method development was performed using homogenized muscle tissue of *Squalius cephalus* (Chub) spiked with target analytes at low ng/g levels. In order to enhance the analytical reliability in complex fish matrices, the method included extensive usage of isotopically labelled surrogate standards. Two different extraction techniques, ultrasonic extraction and pressurized solvent extraction, were compared using different solvent combinations, taking into account two key parameters: absolute recovery (including extract suppression) and reproducibility. At the optimized conditions for LC/MS analysis, a Synergy Polar HPLC column was selected for chromatographic separation, using water and methanol, both containing 0.1% formic acid (v/v), as eluting solvents. The selectivity of the method was assured by highly specific MRM detection, using the two most abundant precursor/product ion transitions for each analyte and surrogate. The developed analytical method was used to assess API concentrations in feral freshwater fish from the Sava River (Croatia).

4.13.P-Tu277 Indoor Environments - Hospitals, Households of Oncology Patients and Others - As a Contamination Source of Hazardous Pharmaceuticals

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Antineoplastic drugs (ADs) used for treatment of cancer are substances with potentially hazardous properties. Assessment of indoor contamination by ADs allows to assess both human exposures and health risks and estimate release of ADs into the environment¹. The present study assessed levels and profiles of surface contamination in the hospital workplaces in the two Central-Eastern European countries, and provided the first research of surface contamination inside houses of oncology patients, hospices and retirement houses. Surface samples were collected in 32 different larger hospitals in the Czech Republic and Slovakia. In addition, repeated samplings of comparable sites were collected from households of oncology patients, as well as in 2 hospices and 3 retirement homes. The wipe samples were analyzed by LC-MS and ICP-MS for analysis of 11 ADs (cyclophosphamide – CP, 5-fluorouracil – FU, Paclitaxel – PX, Ifosfamid – IF, Irinotecan – IRI, Metotrexat – MET, Capecitabin – CAP, Doxorubicin – DOX, Docetaxel – DOC, Gemcitabine – GEM, Epirubicin – EPI, total platinum – Pt)². **1. 2. 3.** In hospitals, the most contaminated surfaces were floors of administered rooms and 50% of all samples contained both CP and Pt. Levels of ADs as well as frequency of exceedance of the recommended Threshold Guidance Values were comparable in both large hospitals

and smaller patient care units³. Other analyzed ADs were also commonly detected (namely GEM, IF, PX and IRI) but in lower concentrations compared to CP, FU and Pt. In households of oncology patients, contamination of carcinogenic CP was commonly found with maxima 500 pg/cm². Sweat of the patients is a important medium for the spread of the contamination. The studies in hospices and retirement homes indicated low exposures but pointed to potential long-lasting contamination by ADs. A monitoring of indoor environments documented wide-spread contamination by ADs. Maximum contamination in hospitals repeatedly exceeded 10 ng/cm², which calls for remedial actions and nurses administering chemotherapy were the main cohort in risk³. Carcinogenic CP was also found in households of oncology patients. The contamination levels in households were comparable to medians hospitals but potential exposures and risks to family members are only temporal⁴. Nevertheless, administration of chemotherapy, which is currently moving from (controlled) hospital healthcare to households³ may represent an issue, which should be further explored in detail. REFERENCES: [1] Nassour, C. et al. 2020. <https://doi.org/10.1007/s11356-019-07045-2>; [2] Bláhová, L. et al. 2021. <https://doi.org/10.1007/s00420-021-01671-5>; [3] Doležalová, L. et al. 2021. <https://doi.org/10.1007/s11356-021-17607-y>; [4] Bláhová, L. et al. 2021. <https://doi.org/10.1186/s12302-021-00544-5>. (Acknowledgement – Supported by the Ministry of Health of the Czech Republic, grant No. NV18-09-00188).

4.14 Predicting the effects of global change on the emission, fate, effects and risks of chemicals in aquatic ecosystems: international collaboration and innovative approaches

4.14.T-01 The ECORISK2050 Project: Predicting the Effects of Global Change on the Emission, Fate, Effects and Risks of Chemicals in Aquatic Ecosystems

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By 2050, the world population will reach nine billion people and three quarters of the global population will live in cities. The development path to 2050 will be marked by shifts in land use and weather patterns, and by changes in the way water and food resources are obtained and managed all over the world. These global changes (GCs) will affect the emissions, environmental transport pathways and fate of chemicals, and thus affect the exposure of the natural environment to chemicals. Future changes may also alter the sensitivity of ecosystems to chemical exposure. To help address these issues, the ECORISK2050 project brings together a world leading and interdisciplinary consortium of universities, research institutes, industry and regulatory and governmental authorities to deliver a cohort of Early Stage Researchers (ESRs). The coupled training goals and research objectives of the project are: (1) to assess how the inputs of chemicals from agriculture and urban environments and their fate and transport are affected by different environmental conditions, including those of specific EU regions, and how this will change under GC scenarios in order to assess the likely increase in chemical risks to human and ecosystem health; (2) to identify potential adaptation and mitigation strategies that can be implemented in the short and medium term to abate unacceptable changes in risks, and use the GC scenarios to propose robust implementation pathways, and (3) to develop a set of tools for use by industry and policy makers that allow the impacts of a range of GC-related drivers of chemical risks to be assessed and managed. The project will deliver the next generation of scientists, consultants and industry and governmental decision-makers who have the knowledge and skill sets required to address the changing pressures that chemicals emitted by agricultural and urban activities pose to aquatic systems on the path to 2050 and beyond.

4.14.T-02 Forecasting Emissions of Metals to Urban Aquatic Environmental Using the Shared Socio-Economics Pathways (SSPs)

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Urban environments are hotspots of metal pollution as they are areas comprising: high population density, heavy traffic, construction and demolition sites, industries activities and ineffective waste and wastewater management. Despite the fact that metals are causing harmful effects on the environment, the future outlook of metals emissions, which will be impacted by socio-economic change, climate change and technological development, is uncertain. This means that we have a limited understanding of how metals emissions in cities will change in the future. The shared-socio-economics pathways scenarios (SSPs), developed for scientists to study the future, provide a potential framework for predicting future chemical emissions. The aim of this project was therefore to study how will emissions of metals of current concern for aquatic systems could change in the future in urban environments in three cities: York (UK), Oslo (Norway) and Madrid (Spain). Research comprised four components: 1) review data on metal concentrations in urban environment worldwide, 2) identify metals that currently pose an unacceptable risk by

calculating risk quotients (RQ), 3) to monitor metals with an RQ >1 seasonally in Madrid, York and Oslo; and 4) develop a framework to extend SSPs to the chemical sector and to apply these to metals in the three cities. Data were found on metal concentrations in 9 urban rivers in 7 countries. Concentrations ranged from 0.03ug/L to 7 433 ug/L for iron in Manisa city in Turkey. Risk quotient was calculated for each continent. In Europe, 7 metals had a risk quotient above 1 (aluminum, copper, lead, mercury, nickel, silver, zinc) compared to 4 in Asia and 1 in North America. Aluminum, copper, nickel, silver and zinc were measured in Madrid, Oslo and York in spring and summer 2021. Expect for nickel and for copper in spring in Oslo, concentrations of aluminum, copper and zinc were systematically detected above predicted no-effect concentrations (aluminum: 4.4ug/L; copper: 0.44ug/L; zinc: 1ug/L) in the three cities. To be able to study how will metals emissions change in the future, a 4-steps framework was developed specifically to adapt SSPs storylines to chemicals. The framework allows the consideration of all socio-economics drivers of the society, allowing a broader understanding of metals in societies essential for decisions-making on metal pollution. The next steps of this research will be to develop priority chemicals emissions in the future for Madrid, Oslo and York using the framework.

4.14.T-03 Combined Effects of Heatwaves and the Herbicide Terbutylazine on Natural Zooplankton Communities: Implications for Global Climate Change

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Understanding the impacts of climate change and its extreme events, particularly heatwaves, on aquatic ecosystems requires exploring interactions with other anthropogenic stressors, such as pesticides. The herbicide terbuthylazine serves as alternative pesticide to the in the EU banned atrazine, yet, it demonstrated lethal effects on phytoplankton communities, leading to indirect starvation of zooplankton. We exposed natural freshwater zooplanktonic communities to a simulated heatwave (one week duration) and the herbicide terbuthylazine (15 µg/L) in different combinations of the two stressors. When applied individually, the heatwave increased the total abundance of zooplankton (3-fold higher). The herbicide application, however, did not affect the total abundance of the zooplankton. In contrast, the combination of the heatwave and terbuthylazine had no impact on the community, suggesting antagonistic effects of the co-occurring stressors on zooplankton. Yet, the terbuthylazine application affected the zooplankton indirectly and led to a community composition change. Future research should further investigate thermal and chemical stressor applications in sequence, as heatwaves are likely to increase in frequency in the future.

4.14.T-04 Sensitivity of Changing Macroinvertebrate Communities in a Warming World

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Taxa have varying sensitivities to chemicals, and species non-exchangability means different assemblage compositions vary in sensitivity. In natural ecosystems, species composition of assemblages varies spatially in response to biogeographic factors, including temperature. Climate change will increase temperature and hence composition meaning sensitivity of assemblages to chemicals may shift. Here we explore the potential consequences of future climate change on the sensitivity of freshwater macroinvertebrate assemblages to chemical stressors using Great Britain as a case study. Macroinvertebrate assemblages under end of century (2080-2100) and baseline (1980-2000) climate conditions for were predicted for 753 sites. Differences in taxonomic composition were compared with Jaccard's index and changes to taxa identified. Freshwater macroinvertebrate toxicity data were collated for 40 chemicals grouped into insecticide, metal or narcotic. The hierarchical Species Sensitivity Distribution (hSSD) model was then used to predict the sensitivity of untested taxa using relatedness within a Bayesian approach. Species sensitivity distributions and HC₅ values were then calculated for each assemblage under baseline and future scenarios. All three climate scenarios resulted in a decrease in Jaccard's similarity compared baseline conditions (range of 0.76-0.80) and 55 taxa were lost from all sites under climate change conditions. The shift in taxonomic composition due to warming resulted in different effects depending on chemical identity and type. Under all three future climate scenarios, metals had a significant decrease in mean HC₅ value compared to baseline, indicating that macroinvertebrate assemblages became more sensitive to metal stress. Similar shifts in average sensitivity were not observed for insecticides and narcotics. Climate change shifted the composition of freshwater macroinvertebrate assemblages and their sensitivity to chemical stress. Climate-induced change in assemblage sensitivity meant on average assemblages were more sensitive to metal exposure under all future climate scenarios. Climate change also resulted in increased between-assemblage variation in the sensitivity of macroinvertebrates to all three chemical stressors, indicating that specific sites and their associated assemblages were exhibiting differential changes in chemical sensitivity. Therefore, site specific spatial variation in the effects of climate change should also be considered.

4.14 Predicting the effects of global change on the emission, fate, effects and risks of chemicals in aquatic ecosystems: international collaboration and innovative approaches (Poster)

4.14.P-We224 A Modelling Framework to Project the Future Sensitivity of Aquatic Invertebrates to Chemicals With Different Mode of Action

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Climate change has already modified several meteorological and hydrological parameters. However, the effects of the changing climate are expected to become more severe in the near future (2050). Changes in water discharge, increasing number of days

with low flow and increased water temperature will force many aquatic taxa to change their actual distribution to find suitable habitats. In the present framework, we use hydrological models able to project changes in several hydrological parameters combined with climate projections to predict changes in aquatic invertebrates' distribution at the European level. In order to simulate present-day natural flow regimes and future flow regimes under climate change, the global hydrology model WaterGAP3 is applied. All calculations for current and future conditions (2050s) are carried out on a 5' × 5' European grid. Calculations of future hydrological conditions are based on the Intergovernmental Panel on Climate Change (IPCC)'s Fifth Assessment scenarios, and predictions of future water availability are generated using the WaterGAP3 modelling framework. This modelling approach was subsequently exploited to derive projections on how the predicted changes in hydrological conditions are likely to shift present-day distribution of aquatic invertebrates, because of a modification of their habitat in terms of hydrology and climatology. Present and future freshwater macroinvertebrates distribution was modelled using Bioclimatic Envelope Models (BEMs) based on an ensemble forecasting framework built on six algorithms (generalized linear models, flexible discriminant analysis, classification tree analysis, random forest, multivariate adaptive regression splines, and maximum entropy modelling). Finally, we used the projected future species distribution to study how the sensitivity of aquatic invertebrates to chemicals with different modes of action is likely to change by 2050. To do that, we used species traits as common currency to calculate species sensitivity to chemicals with different mode of action. Overall, the framework we are presenting, is composed by several steps and combined numerous modelling approaches. It brings together experts from different disciplines, and it proposes, for the first time, a new tool able to give indications on how climate change is likely to shift the relative portion of sensitive aquatic invertebrates to chemicals with different modes of action.

4.14.P-We225 Incorporation of Direct and Indirect Climate Change Effects Into a Probabilistic Pesticides Risk Assessment: A Northern European CASE Study

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Weather patterns in Northern Europe are projected to change with increased temperature and precipitation by 2050. These climatic changes can potentially affect the transport and degradation of pesticides in the environment. Moreover, agricultural practices are expected to change such as application patterns. The pesticide application rates are expected to be altered due to an increased frequency of potential plant disease and insect pests. In this study, we have used a Bayesian network to better integrate these potential direct and indirect climate change effects on the pesticide exposure in a probabilistic approach to risk assessment. The Bayesian network serves as a meta-model that incorporates predictions from a pesticide fate and transport model WISPE - World Integrated System for Pesticide Exposure. We ran the exposure prediction model for specific environmental factors linked to a representative Norwegian study area with specific soil and site parameters, as well as chemical properties under different scenarios of climate model projections (HADCM3-Q0 A1B SMHI RCA3 & ECHAM5-r3 A1B SMHI RCA3) and pesticide application pattern. The Bayesian network links the pesticide exposure predictions derived for the study area to effect distributions derived from toxicity tests to predict the risk quotient distribution. Thus, this approach considers both direct climate change impacts (on pesticides fate and transport) and indirect effects (on pesticide application). Compared to traditional (deterministic) risk assessment methods, this probabilistic approach can better account for uncertainty associated with climate projections.

4.14.P-We226 Enabling Forecasts of Environmental Exposure to Chemicals in European Agriculture Under Global Change

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Agricultural practices emit a wide range of organic chemicals into the environment through the use of pesticides, veterinary treatments, chemicals in biosolids used as soil amendment, and chemicals in wastewater used in irrigation. Furthermore, the 21st century will be marked by a litany of global changes, including climate change, shifts in agricultural practices in response to climate change/shifting demand, and transitions toward a circular economy. These global change forcings portend changes in how and what types of chemicals will be emitted, their persistence and transformation, and the fate and transport mechanisms acting on them, all of which will drive changes in environmental exposure to these compounds. However, current methods do not consider these interlinked forcings in forward-looking exposure assessments. With a focus on European agriculture, we review existing literature to critically examine how global change drivers of agricultural chemical exposure may impact the 1.) emissions, 2.) persistence and transformation, and 3) fate and transport of chemicals in the coming decades. Key knowledge gaps include how the use of reclaimed wastewater for irrigation may change due to increased stress on water resources, how the use of veterinary medicines and pesticides may change under changing pest pressures, uncertainty surrounding changes in microbial degradation of chemicals, and a lack of modelling studies capturing projected changes in extreme weather events and the impact of this on chemical transport. To address these knowledge gaps, we advocate for a holistic approach towards agricultural chemical exposure assessment that considers the identified global change drivers. Similar to the IPCC's Shared Socio-Economic Pathway (SSP) approach that considers scenarios for future development and associated GHG emissions, we propose analysis of four Agricultural Chemical Exposure Scenarios (ACES) including one optimistic, one moderate, one severe, and one extreme scenario of the evolution of global change forcings in the context of chemical exposure. Such an analysis would bound possible agricultural chemical exposure futures and inform actions that could be used to mitigate exposures from exceeding threshold levels under future conditions. Enabling forecasts of environmental exposure to chemicals in agriculture is a vital step in ensuring the global-scale changes that affect agriculture over the 21st Century occur in a safe and sustainable manner.

4.14.P-We227 Testing the Influence of Temperature on Dissipation Rates of a Trace Mixture of Pharmaceutical Compounds in Freshwater Sediments

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The dissipation rates of pharmaceuticals are important considerations in chemical risk assessment, particularly under future environmental conditions where different European regions could experience elevated temperatures for longer periods, potentially affecting the kinetics of pharmaceutical dissipation. Freshwater sediments are also an understudied environmental compartment in this context with emissions of pharmaceuticals to freshwater bodies often occurring as a complex mixture of contaminants which can partition to sediments where they might persist and pose a risk. Therefore, the objective of this study was to investigate the dissipation kinetics of a pharmaceutical mixture in different freshwater sediments across a temperature gradient relevant to future environmental conditions to determine how temperature may drive changes in chemical dissipation rates and half-lives. A 96-day dissipation study was performed by collecting 6 freshwater sediments with contrasting physicochemical properties from across North Yorkshire, UK. The sediments were sieved to 2mm and allowed to equilibrate at either 12, 15, 20, 25 or 30°C for 10 days, before being spiked with a 6-compound pharmaceutical mixture (amitriptyline, atenolol, cimetidine, diltiazem, mefenamic acid and ranitidine) at a nominal concentration of 16.6 ng/g sediment (dry weight) for each study compound. Test replicates (n=18 per sediment and temperature) containing 3g spiked sediment and 4.5mL of field site water were then incubated in the dark at a respective test temperature for 96-days. Sub-samples (n=3) were collected on Day 0, 7, 12, 24, 48 and 96 to undergo a 3-cycle solvent extraction followed by UHPLC-MS/MS analysis to determine chemical concentrations of each study compound. Preliminary results showed nearly complete dissipation (e.g., DT95) of atenolol, mefenamic acid and ranitidine after 48 days at 12°C in all sediment types. At 30°C, complete dissipation after 48 days was observed for all compounds except amitriptyline, which notably showed similar persistence at 12°C and 30°C in three of the sediment types. Data for the remaining test temperatures and timepoints will help inform the dissipation kinetics and chemical half-lives of the pharmaceutical mixture. The influence of temperature and contrasting sediment composition on the dissipation kinetics of the pharmaceutical mixture will be discussed under the theme of supporting chemical risk assessment of pharmaceuticals.

4.14.P-We228 Atmospheric Transport of Compounds of Wastewater Origin Into Remote, Upland Lakes in Ireland: A Consequence of a Changing World?

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Persistent organic pollutants of industrial origin are transported long distances in the atmosphere and deposited into aquatic and terrestrial ecosystems in remote regions. However, there has been little research to determine whether less persistent, polar compounds such as pharmaceuticals, drugs of abuse and constituents of foods and beverages are transported in the atmosphere. The objective of this research was to evaluate whether compounds of wastewater origin are present in four remote, upland lakes in Ireland that receive loadings from atmospheric deposition. In passive samplers deployed in the lakes for between 60 and 68 days, six compounds were consistently detected at levels that could be quantified, including the cannabinoid metabolite, tetrahydrocannabinol carboxylate (THC-COOH), caffeine, acetaminophen, and the artificial sweeteners, sucralose and saccharin. Cocaine, ketamine and tramadol were also detected in passive samplers deployed in some of the lakes, but at levels below the limits of quantitation. Except for caffeine, the estimated concentrations of the compounds in lake water were all below 150 ng/L. The highest concentrations of the target analytes were detected in two lakes located on the east coast of Ireland where weather patterns are dominated by westerly winds. These data are consistent with the transport of these compounds in aerosols originating from wastewater treatment plants in continental Europe and/or the U.K. and deposition in rainwater into the catchments of the remote lakes in Ireland. Continued monitoring is warranted to determine if an increase in the use of drugs of abuse and pharmaceuticals, plus changing weather patterns are contributing to contamination of remote lakes.

4.14.P-We229 Training As a Strategy to Mitigate the Impact of Climate Change

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“Connect science to politics and the population and provide health and climate change education for healthier cities” is one of the key Message of Rome International Charter on Health and Climate Change, addressed to policy makers and all the stakeholders involved in the management of climate changes. Cities must identify strategy and make intervention for adaptation and mitigation of climate change indeed actually they are heavily affected by climate change such as intense precipitation and heat waves; furthermore people must be educated about climate change, their effect on human health. The Italian Project, “Adaptation and mitigation to Climate Change: urban interventions for the promotion of Health – CLIM ACTIONS” funded by the Ministry of Health and coordinated by the Department of Epidemiology of Latium Region, aims to identify strategies and interventions to mitigate the urban heat island and air pollution, promoting health benefits for the population in the urban context of cities across Italy, Turin, Genoa, Bologna, Rome, Bari and Palermo. One of its activities is dedicated to share the knowledge about climate changes and their potential risks for human health to health professional sectors, stakeholders and citizens. The training is being realized through a on line course performed with the e-learning tools available on the platform of Istituto Superiore di Sanità (Eduiss). This course, based on scientific evidences covers following issues, climate changes and their impact on ecosystems and human health; urban heat islands, air pollution effects on citizens, and the role of green and blue spaces on human health, wellbeing and on mitigating climate change effects. This course will be based on Problem Based Learning PBL, a teaching method in which a simulating real problems used as the vehicle to promote student learning of concepts and principles as

opposed to direct presentation of facts and concepts. It will be dedicated to health professional sectors, stakeholders and everyone is interested to deepen the knowledge on climate change effects on human health.

4.14.P-We230 Human Health Through Environmental Protection: An Example of Management of Environmental Emergencies

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The environmental emergency affects both water, air and soil and requires the immediate activation of operational structures, which carry out environmental protection interventions. All European countries are committed to environmental protection and their policies are directed towards sustainable development. One of pillars of Green Deal is the protection of the health and well-being of citizens as well as the ecosystems in which they live. In order to face the challenge related to the protection of the health and safety of citizens and ecosystems, it is essential to act promptly and avoid that (not appropriately managed) events can cause the environmental degradation, determining direct deterioration of the quality of life and health conditions of citizens. It is essential to establish a close collaboration between citizens, institutionally responsible authorities, research bodies and the industry, in order to reach shared solutions. Environmental emergencies often have impact on human health both in terms of acute/chronic effects, including psychological effects, and of socioeconomic consequences that have implications on health. In case of emergencies occurring in Italy, the Italian Institute for Environmental Protection and Research (ISPRA) ensures scientific technical support to the responsible authorities. To ensure the effectiveness of the response, ISPRA has adopted a *Regulation for the activation in emergency*, which provides for integrated and synergistic management tools that are carried out through the activation of an Operational Network of Representatives of the Regional Agencies. This exchange network guarantees the circulation of information and experience, allowing the entire territory to properly address the occurrence of environmental criticalities, exploiting the best experiences and available skills. From ISPRA's activity, reports have emerged containing the description of the events and the operating procedures for activation with the Operational Network but also information on the most involved areas (43% surface water bodies) or on the type of predominant event (fire). By focusing on an actual case study, the goal of the research is to confirm that the scientific community addresses to all public and private components so that a process, currently unstructured, is promoted which pushes the private sector to invest in order to reduce the environmental and economic impacts of production, and the public authorities to propose solutions.

4.14.P-We231 Development of Chemical Emission Scenarios Using the Shared Socio-Economic Pathways

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The widespread use of chemicals has led to significant water quality concerns, and at the moment, the use of chemicals is still increasing. Henceforth, there is an urgent need to understand the possible future trends in chemical emissions to water systems. This paper proposes a general framework for developing emission scenarios for chemicals to water using the Shared Socio-economic Pathways (SSP), based on an emission factor approach. The proposed approach involves three steps: (i) identification of the main drivers of emissions, (ii) quantification of emission factors based on analysis of publicly available data, (iii) projection of emissions based on projected changes in the drivers, and emission factors. The approach is tested in Europe for five chemical groups and at a national scale for five chemicals representing pharmaceuticals, pesticides, and industrial chemicals. The resulting emission scenarios show widely diverging trends of increased emissions by 265% for ibuprofen in SSP3 to 75% decrease for diclofenac in SSP1 by 2050. While emissions typically decrease in SSP1 (sustainable development), they follow the historical trend in SSP2 (middle-of-the-road) and show an increase in the regional rivalry scenario SSP3 for most selected chemicals. Overall, the framework allows understanding future chemical emissions trends as a function of socio-economic trends. These scenarios can be used to model aqueous emissions to support risk assessment. While the framework can be easily extended to other pharmaceuticals and pesticides, it heavily leans on the availability and quality of historical emission data and a detailed understanding of emission sources for industrial chemicals.

4.15 Recent advances in Bioremediation and Phytoremediation of contaminated areas

4.15.T-01 Unveil Microbial Community Structure of Terrestrial Microbial Fuel Cells

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Soil contamination has great impact on soil fertility, plant growth, food quality and ultimately affect human health. The contamination of agricultural and industrial lands has direct and indirect effects on human health and ecosystem. Bioremediation is considered as an eco-friendly and economical approach and microbial fuel cells (MFCs) attracted much attention as an energy saving bioremediation tool. In particular, terrestrial MFCs (TMFCs) have many advantages like eco-friendly, cheap, safe, clean, easy operation and sustainability. TMFC is a promising system for the bioelectricity production as well as the soil remediation. In TMFCs, the anode is buried in water saturated soil connected via external circuit to cathode on top of soil. In the buried anode, a biofilm is generated and organic matter is degraded (oxidation) by exo-electrogen bacteria capable to donate electrons which are then simultaneously transferred to the cathode compartment via an external circuit. Protons produced by exo-electrogen bacteria in the anode, diffuse through soil to the cathode where oxygen is primarily used as the oxidant, due to its abundance and high

reduction potential. The flow of electrons generate electricity. Several studies reported that biofilm at anode is typically enriched by bacteria belonging to *Geobacter*, *Skermanella*, *Desulfucapsa*, *Desulfobulbus* and *Desulfuromonas* genera. In this work, we have analyzed the prokaryotic community structure of a TMFC amended with compost in three different points: anode, cathode and bulk soil at 2 months from the experimental set-up. Control TMFCs consisting of only soil were also performed. Electric measurements (power and voltage) were evaluated daily; moreover, abundance, viability, activity and structure (NGS analysis of the 16S rDNA) of the microbial community were evaluated at the start and end of the experiment. The results showed significant differences in the compost-TMFC microbial community among anode, cathode and bulk soil. At the anode, a higher cell abundance and activity than cathode and bulk soil were found. Moreover, an increase in the *Bacillus*, *Clostridia*, *Bacteroidia* and *Deltaproteobacteria* and a decrease in *Alphaproteobacteria* and *Actinobacteria* genera were observed at 2 months. *Gammaproteobacteria* were also reduced in presence of compost at the cathode and bulk soil. The genus *Geobacter*, an exoelectrogen bacteria, was found at anode and bulk soil in presence of compost. Interestingly, microbial soil activity was positively correlated to voltage and power generated by the MFCs.

4.15.T-02 Effects of Contrasting Biochars on Copper Speciation, Bioavailability and Toxicity in Trace Element-Contaminated Soil

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Chromated copper arsenate (CCA) contaminated soils are commonly found at former wood impregnation sites and pose great risks to human and environmental health. Biochar has been proposed as an adsorbent for mitigating trace element toxicity in contaminated soil. We investigated the effects of fourteen biochars on the dynamics of copper speciation (Cu ion selective electrode), copper bioavailability (whole-cell bacterial bioreporter), and soil toxicity (seed germination and *Arthrobacter globiformis* solid-contact assays) in CCA contaminated soil during a controlled 56-day experiment. Specifically, our study focused on the dynamics of Cu speciation and bioavailability, as Cu was previously found to be the main toxicant in the studied soil (Tardif et al., submitted manuscript). Biochars were derived from bone meal (Bone), *Miscanthus* (M), *Miscanthus* straw (MSP), oil seed rape straw (OSR), rice husk (RH), sewage sludge (SS), softwood (SWP) and wheat straw (WSP) and were pyrolyzed at different temperatures (550, 700, 850 or 950 °C). OSR550 and OSR700 showed the best performance and increased soil pH while decreasing the free Cu²⁺ activity by 78%. All biochars except SWP550 significantly decreased bioavailable Cu ($p < 0.05$). Moreover, biochar treatments reduced soil toxicity as indicated by increasing plant shoot and root growth and increased bacterial dehydrogenase activity as determined by the *Arthrobacter globiformis* solid-contact assay. Soil toxicity was best predicted by the free Cu²⁺ activity. Hence, the free Cu²⁺ activity showed significant correlations (Spearman) with plant shoot length ($r = -0.86$, $p < 0.001$), root length ($r = -0.58$, $p < 0.05$) and soil toxicity determined by *Arthrobacter globiformis* assay ($r = 0.79$, $p < 0.001$). Bioavailable Cu could also well predict soil toxicity, whereas water-extractable Cu was a relatively poor soil toxicity predictor. In conclusion, our study showed a great potential of biochar derived from oil seed rape straw pyrolyzed at 550°C for remediation of chromated copper arsenate contaminated soils and soil toxicity mitigation was best explained by reduced free Cu²⁺ activity. Our study provides a basis for future development of effective custom biochars for Cu stabilization in trace element-contaminated soils and highlights the *Arthrobacter globiformis* solid-contact assay as a promising technique for evaluating soil toxicity dynamics during soil remediation processes.

4.15.T-03 Towards a Practical Guideline for Applying Carbon-Based Materials for Soil Remediation

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Biochar application for immobilizing heavy metals and Polycyclic Aromatic Compounds (PACs) in contaminated soils has been a field of active research for the last decade. However, there is still a lack of guidance to put what has been demonstrated in the lab into practice. As not all materials are suitable for the same type of contaminant and/or application, a major difficulty for practitioners is the selection of suitable sorbents from the variety of carbon-based materials available (including biochar and activated carbon), which widely differ in their physical and chemical characteristics. A generic assessment of biochar quantities needed for a specific remediation application at full scale is not possible, due to these specific material properties, as well as differences in contamination scenario and soil properties. Here we aim to provide a guideline to evaluate the applicability of carbon-based materials for heavy metal and PAC contaminated soil remediation for scientists and practitioners alike. The guideline is built on literature-based as well as experimental work: we initially carried out a literature review collecting knowledge on the influence of feedstock, production temperature and possible modifications on a material's physical and chemical characteristics. We further conducted expert interviews with practitioners and regulators in Austria. Thereafter, laboratory experiments complemented the knowledge collected. For our experimental work, we used eleven carbon-based materials and a total of ten anonymized contaminated soil samples from existing remediation sites containing varying amounts of arsenic, antimony, cadmium, zinc, lead and PACs. The information gathered informed a table that provides contaminant-sorbent combinations suitable for immobilization, as well as an estimate of sorption affinity ($\log K_D$) that could be expected for a type of sorbent and a given contaminant. Overall, we present a workflow to determine site-specific suitability and tailored amendment rate of biochar for soil remediation. Our workflow will help decision makers assess whether biochar can be used at a given site.

4.15.T-04 Natural Bioremediation and Bioaugmentation of Soils Conditioned With Foaming Agents

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The mechanized excavation of tunnels produces large quantities of excavated soil that contains residual concentrations of foaming agents used to modify the mechanic and hydraulic behavior of soil. The classification as a by-product or as waste of excavated soils depends not only on the residual presence of known contaminants (e.g. metals, hydrocarbons, etc.), but also on the presence of other chemical substances such as anionic surfactants which are the main components of foaming agents (5-50%). For these substances there are no legislative limits, however their presence at certain concentrations can determine a potential toxicity of the excavated soil. Our research reports the main results on the biodegradation of the anionic surfactant sodium lauryl ether sulphate (SLES), with a focus on the recently selected microbial consortium capable of degrading anionic surfactants in a few hours and on its use in bioaugmentation applications. For this reason, three different experiments were set up: microcosm (to assess SLES biodegradability), isolation (to identify and characterize a bacterial consortium capable to degrade SLES) and bioaugmentation (to test if adding the bacterial consortium identified to spoil material enhanced the removal of the anionic surfactant residues) experiment. The main results of our research showed that the natural attenuation (with no human intervention) of SLES is expected due to natural microorganisms present in the excavated soil (28 days), but long degradation times cannot always meet the construction site requirements. This can be the case of tunneling for a metro in a city or through mountains along a sea coast, where a prolonged storage is not possible because of lack of space and the spoil material would be forced to be considered a waste. The identification of a natural bacterial consortium capable of promptly (9 hours) degrading SLES has significant potential for bioaugmentation purposes, permitting a shortening of the storage time for soil excavated with foaming agents at construction sites and a safe promotion of the subsequent reutilization of the soil for different purposes. In fact, the bioaugmentation experiments of the spoil material with the bacterial consortium previously identified, significantly improved (six times) the degradation time of SLES (DT₅₀ 1 day) compared with natural attenuation (DT₅₀ 6 days), ensuring a safe by-product and saving execution time and overall costs for the tunneling industry.

4.15.T-05 Assessment of Organic and Inorganic Contaminant Removal in Contaminated WATER by Mycofiltration, Through a Fixed Bed Column Approach

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Mycofiltration is an environment friendly technology which involves the treatment of contaminated water by passing it through a network of fungal mycelium. Fungal mycelia are said to employ mechanisms such as biosorption in the remediation of pollutants/xenobiotics. The current study seeks to conduct mycofiltration via a continuous fixed bed column approach. This allows assessment of the effect of various parameters such as inlet concentration, flow rate, pH, and column bed depth on the sorption capacity of the mycofilter in a continuous treatment process. The resulting breakthrough curves generated will thus give a representation of the pollutant-effluent concentration vs time profile, whilst kinetic models will evaluate column performance and predict scale up. A mycofilter of the *Pleurotus* fungal species is currently being cultured. Various substrates (hay, thatching straw, bamboo wood chips and aspen wood chips) have been prepared through cutting into small particles, soaking in 2 % (w/v) calcitic lime and pasteurisation, prior to mixing with *Pleurotus ostreatus* spawn 10 % (w/w) in 140 x 170 mm polyethylene bags. The bags were incubated in a dark, humid chamber to allow for substrate colonisation by mycelium, for a period of 14 days. Upon observations, degree of mycelium colonisation from highest to lowest: thatching straw > hay > aspen wood chips > bamboo wood chips. To create the mycofilters, the pre-myceliated substrate will be loaded into acrylic columns (1 x 10 cm), at various bed heights. Mycofilters will be further incubated for 7 days. A food colour solution will be passed through the columns at different flow rates, filtrate collected at time intervals and absorbances measured. Breakthrough curves will be plotted and amount of contaminant retained at both breakthrough time and saturation time, as well as biomass uptake capacity will be analysed. Study hypothesis: H₀: Mycofiltration via a continuous fixed bed column does not efficiently remove organic and inorganic contaminants in water. H₁: Mycofiltration via a continuous fixed bed column efficiently removes organic and inorganic contaminants in water. Mycofiltration, through a simple inexpensive continuous fixed bed column, will provide valuable information necessary for upscaling from laboratory pilot studies to industrial scale. Mycofilter fixed bed columns could thus possibly be applied in the management of point source pollutants e.g., effluent from wastewater treatment plants.

4.15 Recent advances in Bioremediation and Phytoremediation of contaminated areas (Virtual Only)

6.07.V-01 Assessment of Sunflower Capability in Restoring Soil Contaminated by PCBs and HMs in Microcosm Experiments

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Plant-assisted bioremediation (PABR) is a strategy based on interactions between plants and microorganisms in the rhizosphere, which promotes a reduction in soil contaminants. In fact, the plant root system can stimulate microbial activity in the rhizosphere favoring decontamination processes (biodegradation of organic xenobiotics and removal/transformation of the inorganic ones). In the last few years, several laboratory, greenhouse, and field studies were carried out to assess the effectiveness of PABR technology in recovering multi-contaminated soils. At laboratory scale, microcosm experiments allow studying natural soil

microbial populations under controlled environmental conditions (e.g., temperature, light, humidity, and so on). Recently, sunflower has been proposed for plant-assisted bioremediation purposes. This plant species was demonstrated to be able to absorb various heavy metals (e.g., Zn, Cu, Pb, etc) through its root system. Moreover, sunflower effectiveness in degradation of Persistent Organic Pollutants (e.g., DDT, endosulfan, different chlorinated compounds, and polychlorinated biphenyl-PCBs) was verified recently. In this work, a sunflower microcosm experiment was carried out for 3 months to assess sunflower capability in restoring soil historically contaminated by PCBs and heavy metals (HM). The soil was collected from a survey site located close to Taranto city (Southern Italy). Four different experimental conditions (presence/absence of compost and/or plant) have been set up. Chemical analyses of soil properties (e.g., pH, EC, OC, available phosphorous) and pollutants (PCBs and HMs) were performed at two sampling times (45, 90 days). Moreover, microbial abundance, dehydrogenase activity, and qPCR assays were performed for evaluating the autochthonous microbial community composition, structure, and functioning. Finally, a root exudates screening was carried out on planted pots in order to investigate the potential microbial-plant interactions occurring in sunflower-assisted bioremediation microcosm experiments.

4.15.V-01 Biodegradation Potential of Bacteria Species From Sub Soil of an Artisanal Oil Polluted Site

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The rising incidence of oil pollution caused by artisanal crude oil refining has resulted in the devastation of farmlands, aquacultures, and biodiversity loss. The purpose of this study was to assess the biodegradation capacity of bacteria species isolated from the subsoil of an artisanal oil contaminated site. Crude oil-polluted soil samples were collected in sterile containers from Tombia and Bodo Towns in Rivers State using a soil auger and brought to the laboratory for microbiological analysis. HUB isolation was accomplished by enrichment on Bushnell Haas broth. The average HUB and HUF counts after 72 hours' incubation were 4.6×10^5 CFU/g; 3.6×10^6 CFU/g for Tombia soils, 2.7×10^7 CFU/g and 2.5×10^7 for Bodo soils and 3.5×10^5 CFU/g; 2.2×10^5 CFU/g for Tombia soils, and 2.7×10^5 CFU/g; 3.0 CFU/g for Bodo soil respectively. Colorimetric method with the redox dye, dichlorophenol indole phenol (DCPIP) was employed for degradation screening. *Clostridium* spp, *Pseudomonas* spp, *Actinomyces* spp, *Fusobacterium* spp, and *Bifidobacterium* sp. were the HUB identified while *Candida glabrata*, *Microsphaeropsis arundinis*, *Neosartorya fischeri*, *Aspergillus niger*, *Aspergillus lentulus* were the HUF identified. The study's findings show that the contaminated site has microorganisms with significant biodegradation capacity, and it can be used as an eco-friendly and cost-effective strategy for bioremediation of severely polluted sites.

4.15.V-02 Long Alkane Degradation by Bacteria From Crude Oil Polluted Site

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Petroleum hydrocarbons have been used extensively as a source of fuel. Due to this, exploration has gone on continuously with recurring oil spills within the Niger Delta region of Nigeria. In this study, soils from two different sites of crude oil pollution were subjected to tests to observe the degradation of long alkanes by bacteria found natively within these sites. Soil samples were collected and subjected to physicochemical tests using standard methods. Microbial counts were determined using enrichment and vapour-phase methods for enumeration of hydrocarbon utilizing bacteria. Bacteria were isolated and characterized using biochemical tests. The bacterial counts for the enrichment phase ranged from 5.23 to 185.0×10^4 cfu/g for the BSP soil, while it was from $4.1 - 747.0 \times 10^4$ cfu/g for the TSP soil and for the vapour phase method, 2.33×10^5 cfu/g for the paraffin oil setup for the BSP soil while it was 3.55×10^5 cfu/g for the paraffin oil setup for the TSP soil. In all soil, there was no growth of degraders for the paraffin wax setup. The isolated bacterial species found responsible for the degradation of paraffin (both oil and wax) were characterized and identified to belong to the following genera: *Acinetobacter*, *Arthrobacter*, *Pseudomonas*, *Rhodococcus* and *Bacillus*. It known that these bacteria may be employed in biodegradation set up to clean up the long alkane fractions in contaminated areas effectively as they utilized paraffin oil.

4.15 Recent advances in Bioremediation and Phytoremediation of contaminated areas (Poster)

4.15.P-Th140 Phytoremediation Potential of Contaminated Shooting Range Soil

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Contamination of shooting ranges is an important environmental issue, and it is important to manage and control this pollution in the shooting ranges. The range contributes significantly to the contamination with heavy metals, especially lead. The aim of the study was test phytoremediation potential using a heavily contaminated shooting range soil in 80 days field study. In this field study two species (*Trifolium pratense* L. and *Medicago sativa* L.) were used for comparison of their phytoremediation efficiency. The concentration of lead in the soil in the most contaminated area of the shooting range was up to 54.5 g kg^{-1} . Significantly higher concentrations of heavy metals were found in the aboveground parts of both plant species when exposed to the most polluted shoot soil. The growth *M. sativa* was no so sensitive as *T. pratense* - significantly lower biomass of *T. pratense* was observed in plants grown in the soil of the shooting range compared to the control while *M. sativa* did not show significant growth changes under the exposure to contaminated soil., resulting in lower plant growth.

4.15.P-Th143 Heavy Metals Vermiremediation Potential Using *Eisenia fetida* at Different Sewage Sludge Treatments in Soil

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Sewage sludge production is constantly growing across the world, while sustainable sewage sludge (SS) disposal is a major environmental problem. In European Union the predominant valorization of sewage sludge is agricultural or forestry reuse for soil fertilization or land reclamation/restoration. Sewage sludge secondary use in agriculture is actively promoted for soil quality improvement due to its high amount of organic matter and nutrients. Along with many advantages of SS application to soil, it has drawbacks such as non biodegradable heavy metals, various organic contaminants, pathogenic organisms. Soil contamination with heavy metals not only endangers ecosystems, but also poses human health risk. Biological remediation of contaminated soil offers significant economic, energy, environmental and social advantages compared to traditional (physico-chemical) methods. Earthworms and their products (vermicompost) have emerged as a potential environmentally friendly option among biological remediation solutions. The purpose of this study was to assess the potential of the earthworm *Eisenia fetida* to remove heavy metals in SS supplemented soil. The vermiremediation process was performed at different SS soil treatments (0-200 kg/ha) using adult earthworms. As a result of vermiremediation, soil heavy metal concentrations were significantly reduced. Higher than 80 % vermiremediation efficiency was reached for a few heavy metals (Ni, Co, Mn), and the bioconcentration factors were as follows: Zn>Co>Cu>Ni>Mn>Cr. In addition, vermiremediation affected soil quality: soil pH was stabilized, organic matter mineralization quickened, K and Mg concentrations have slightly decreased, and the content of Ca and key nutrients (P, S) has increased. The greatest potential for vermiremediation was reached in SS soil treatments of 25-50 kg/ha. It is important to note that higher SS concentrations (> 100 kg/ha) in soil may limit this method's use due to possible lethal effects on earthworms and growth retardation. Altogether, the study suggests that vermiremediation using *Eisenia fetida* might be a sustainable, successful and environmentally friendly management strategy for stabilizing SS amended soil, reducing toxic effects and converting it suitable for safe use in agriculture.

4.15.P-Th144 Two-Way Reuse of Sewage Sludge Through Co-Composting With Sludge Derived Biochar

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This study is a part of the international project RESIDUE in the framework of the EU PRIMA programme with a special focus on Mediterranean area. The goal of this study is to investigate new ways of recycling of sewage sludge as a product of waste water treatment for agricultural use in Israel with a possibility of applying it to other Mediterranean regions with organic matter scarcity. For that purpose, we are testing the co-composting of sewage sludge with the biochar derived from local waste streams. Co-composting of biochar with organic matter is a promising way to turn waste materials into agricultural substrates. Our WG Geoecology achieved good results with co-composting of green waste with wooden biochar in previous projects (TerraBoGa, CarboTIP). At first, the input materials for the co-composting process were selected in Israel. The cooperation with Compost Or, which is the biggest sewage sludge recycling facility in Israel, allowed us to use representative sewage sludge input material, which is collected through over urban waste water treatment plants in Israel. Part of the recycled and composted sewage sludge is also used as an input material to produce biochar (carbonizate) at the Earth Biochar (subsidiary of Compost Or) facility, which is used for example as a soilless substrate for plants. We decided to take that sewage sludge derived biochar to enable the two-way reuse of sewage sludge through co-composting. For that purpose, an on-site composting trial was established at Compost Or facility with variants of 10, 20 and 30 vol.-% biochar in the composting mix. The pre-tests with biochar produced by WG Geoecology from composted sludge at different temperatures (500/600/700°C) showed positive results on biochar stability (H/C, O/C – ratio) and high nutrient contents. These biochars will be compared to biochar produced by Earth Biochar, especially regarding to increased heavy metal content due to input material properties observed in the biochars produced by WG Geoecology. The resulting composts will be characterized on basic physico-chemical parameters, heavy metals, PAH and plant-available nutrients. Leaching tests will be performed to investigate the mobility of nutrients and contaminants in the biochar-composts and in the biochar-compost applied to local soil. These composts will be applied later to Israeli soil in a greenhouse and lysimeter/field trial to assess soil quality improvement.

4.15.P-Th145 Enhanced Natural Attenuation Mediated by Autochthonous Bacteria for Recovering Cr(VI)-Polluted Groundwaters

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The presence of Cr (VI) in groundwater, at concentrations significantly higher than legal limits, represents a widespread environmental problem. Environmental contamination is mainly related to the incorrect management of process water and by-products coming from numerous industrial processes involving chromium. Groundwater remediation can be conveniently achieved by reducing Cr (VI) to Cr (III), since the latter is less soluble and less mobile through environmental matrices, as well as a hundredfold less toxic for living organisms than Cr(VI). Biological reduction is potentially advantageous in terms of economic and environmental sustainability, especially in the case of large volumes of water. Ubiquitous organisms, characterized by high metabolic plasticity, such as bacteria, are able to detoxify the environment surrounding the cell by reducing Cr (VI). However, these microorganisms are generally heterotrophic and need an external source of nutrients to support their growth and

reproduction, especially in oligotrophic environments such as groundwater. Enhanced natural attenuation can be achieved by supplying suitable amendments that can both support bacterial biomass production and provide electrons for reductive processes. The NGS technologies applied to the 16S rDNA region can provide the structure of the native bacterial community, highlighting changes before and after treatment. This approach also allows investigating a possible amendment-driven enrichment of chromium-reducing bacteria. The capability of indigenous bacterial consortia to remediate Cr(VI)-polluted groundwater was investigated by a bioremediation test carried out at a microcosm scale. Microcosms setup was performed using deep saturated soil and groundwater collected from an industrial site in the territory of Barletta Municipality, where Cr(VI) was detected in groundwater at a concentration of about 140 $\mu\text{g L}^{-1}$. The effectiveness of two different amendments for Cr(VI) removal was tested. Results showed a clear dependence of Cr(VI) decay on the amendment used. Notably, yeast extract (200 mg L^{-1}) provided the highest Cr(VI) removal, in comparison with polyhydroxybutyrate (180 mg L^{-1}), whereas no removal was detected in the not amended control reactors. Bioinformatic analyses are ongoing in order to investigate changes in the bacterial community structure.

4.16 Tire wear and microrubber particles - from problems to solutions (Part I)

4.16.T-01 Modelling Tire and Road Wear Particle Fate & Transport in the Terrestrial and Freshwater Environment With a Global Perspective

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This presentation discusses the refinement and extension of the Tire and road wear particles (TRWP) Distribution Model developed by Unice et al. in 2019. This model provided the first integral catchment-scale TRWP mass balance for the Seine watershed (France/Belgium). The overall objective of the work presented was to provide scientifically convincing TRWP mass balances, by spatially and temporally explicit modelling of both mass balances and concentrations found in the environmental compartments water, soils and sediments, and to do so for three watersheds on different continents. To achieve consistent extensions to watersheds on different continents, spatial(-temporal) data available at a global scale had to be used. Such datasets were identified and accessed, to create a high-resolution (< 1km) hydrology and sediment delivery model using a process-based distributed open source modelling framework. Releases of TRWP were estimated at the country scale using statistical data from various sources, and were downscaled using global spatial datasets of population, settlement type and road networks. The model refinement included the implementation of improved terrestrial fate and transport algorithms to allow applicability under a wide range of climatic conditions. The aquatic fate and transport model was kept unchanged, describing transport and trapping of 4 size fractions of TRWP, 3 size fractions of natural suspended particles and 4x3 aggregates formed by hetero-aggregation. For model validation, new analysis results from surface water samples and samples from sediment traps from the Seine River were used. Successful model applications were developed for watersheds on three continents. Robust mass balances for the Seine are presented, grace to the high model resolution and the additional validation. By comparing the three watersheds, insights are generated in differences between the simulated basins caused by socio-economic, climate and storwater management practices gradients.

4.16.T-02 Estimation of Tyre and Road Wear Particle Emissions in Urban Aquatic Systems

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Tyre and road wear particles (TRWP), which are generated from the contact made between the road surface and the tyre, have gained increasing attention due to the perception that they are making a significant contribution to microplastic contamination of freshwater systems. The considerable amount of particles released to the environment may have toxicological effects on aquatic organisms, due to the chemicals added during the manufacturing process and the adsorption of contaminants present in the environment. Annual TRWP emissions have been estimated for various countries, mainly in Europe. However, only a few studies have simulated the transport of TRWPs through surface runoff to freshwater systems. These have typically explored emissions at the regional level. In this study, we used a temporally and spatially resolved model to estimate tyre and road wear particle emissions in the City of York, UK. The exposure modelling used data on tyre particle generation rates, local weather patterns, land cover composition, wastewater treatment plant characteristics, and the drainage/sewerage to estimate emissions to different points in York's river system at a daily resolution. Average, maximum and minimum emission scenarios were modelled. The total TRWPs emitted into rivers in the City of York summed across vehicle types for the minimum scenario was 41 tonnes/year corresponding to a per capita emission of 0.19 kg/year. A total value of 86.30 tonnes/year and 128.75 tonnes/year, and a per capita value of 0.40 kg/year and 0.60 kg/year was calculated for the average and maximum scenario, respectively. Over the three scenarios, cars presented the highest emission values from the four vehicle types studied, whereas buses showed the lowest. Spatial variance of the TRWP emissions can be observed across the city's regions, where the highest emissions were found in the northeast and northwest of the city. Temporal variance is demonstrated between months, where the peak months in the 2017 simulation were February and October. The results can help identify high locations and times of high emissions (hot spots) enabling more accurate exposure and risk assessment and the targeting of future management approaches, such as improved road runoff treatment.

4.16.T-03 Distribution and Propagation of Tyre Wear in Germany and Fate in the Basin of the River Panke in Berlin

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Tyre wear is considered the largest sources of microplastics in the environment in Germany, as for most industrialized countries. Several studies deal with the propagation of tyre wear via air, rainwater runoffs and water transport. However, only a limited knowledge about the spatial distribution of tyre wear as a starting point for propagation calculations exists. The present study integrates a traffic data and a probabilistic model in order to calculate the distribution of tyre wear along the roads as well as its fate in atmosphere and water bodies for the whole area of Germany. As example, this tyre wear model was applied for the river basin of the river Panke in Berlin. The traffic data model uses the average daily traffic volume (ADTV) in order to calculate the traffic volume for three road categories: rural roads, urban roads and motor ways. It defines specific road segments which are characterized by certain traffic situations such as brake zones at intersections, curve radius, and slope inclination. The probabilistic model assigns each street segment a relative wear intensity, which were adjusted by a constant factor in order to match a given total amount of tyre wear particles and is then submitted to the traffic data model for the calculation of the local distribution of tyre wear particles. The atmospheric transport model uses meteorological data to create maps with a resolution of 100m x 100m for Germany and 2m x 2m for the river basin Panke, respectively. It includes deposition, resuspension, sedimentation, transport and transformation of suspended tyre wear particles. The authors of the present study apply the material flow model STORM for the modeling of the source emissions: of the tyre wear particles in the river basin Panke. The maximum tyre wear load calculated for one discharge point accounts to 4,921 kg/a.

4.16.T-04 Physico-Chemical Property Estimations and Fate Predictions of Methoxymethylmelamines: Tire-Derived Transformation Products

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Hexamethoxymethylmelamine (HMMM) is a polymer crosslinking agent that is commonly used to manufacture tires on an industrial scale. Previous work has demonstrated that HMMM is a ubiquitous contaminant in urban surface waters due to its presence in tire-wear particles and its tendency to be transported into receiving waters during rain events through road runoff. It has recently been seen that this chemical readily transforms into numerous other compounds, several of which have also been detected in the aquatic environment. However, there is limited knowledge about the properties of HMMM, its precursor contaminants, and its transformation compounds. Furthermore, the environmental fate of these compounds is largely unknown, which limits the capacity for completing the required ecological risk assessments. To address these data gaps, this study utilized COSMO-RS solvation theory to estimate the physico-chemical properties of HMMM and its derivatives. The trends between the degradation of HMMM (often through demethoxylation) and the corresponding impact on the physico-chemical properties were established and discussed. Furthermore, the estimated values for these properties (e.g., solubility, vapour pressure, log Kow) were then used as inputs to the Equilibrium Criterion (EQC) fugacity-based multimedia model to predict the likely environmental fate of these tire-derived compounds. Three different emission scenarios were modelled in order to comprehensively assess the predicted chemical fate. These scenarios included: 1) input of the chemical equally into air, water, and soil, 2) input of the chemical into water only, and 3) input of the chemical into soil only. Overall, these compounds were predicted to readily partition into aqueous media, with distributions in water increasing with the loss of methoxymethyl groups. In addition, the persistence of the transformation products of HMMM was predicted to decline with the extent of these transformations. The EQC model predictions indicate that these compounds are subject to overland transport into surface waters. This study provides the first insight into the environmental behaviour and fate of HMMM and its transformation products and contributes to the growing literature on the hazards of organic chemicals present in tires and their environmental transformation products.

4.16.T-05 Evaluating Microbial Degradation of Tire Wear Particle Chemicals in Stormwater

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Growing concern over the ubiquity and potential environmental impacts of tire wear particles, particulate debris produced from the friction between tires and the road surface, has resulted in significant efforts to understanding their environmental fate and behavior over the past decade. Tire wear particle microplastics are chemically complex materials containing rubbers, reinforcing agents, resins, additives, process oils, net vulcanization agents, and other compounds that can leach into the surrounding terrestrial or aquatic environment, with poorly understood toxicokinetic and toxicodynamic properties. Hexa(methoxymethyl)melamine and 1,3-diphenylguanidine have been identified as chemicals that may be partially responsible for acute toxicity and mortality impacts observed in aquatic organisms like coho salmon. However, little information is available about the natural bioremediation potential of these contaminants. The goal of this research was to identify microbes in stormwater, which are frequently exposed to these contaminants, that may be capable of degrading hexa(methoxymethyl)melamine and 1,3-diphenylguanidine. Through selective culturing and subsequent Sanger sequencing, three putative biodegraders (*Bacillus cereus*, *Bacillus spp.*, *Lysinibacillus spp.*) have been identified that are capable of using hexa(methoxymethyl)melamine and 1,3-diphenylguanidine as their sole carbon source, a promising finding that can be used in future research to explore the potential for *in situ* bioremediation of tire wear particles.

4.16 Tire wear and microrubber particles - from problems to solutions (Part II)

4.16.T-06 Challenges and Ways Forward in Assessing the Ecotoxicity of Tire and Road Abrasion in Stormwater Runoff - Introduction to the RoadTox Project

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Recently, awareness and knowledge about tire and road wear particles (TRWP) in the context of environmental risk assessment have significantly increased. Yet, the fate and ecotoxicological effects of tire abrasion outside of communal wastewater treatment systems are insufficiently understood. Furthermore, there is a lack of data about how tire abrasion interacts with other pollutants from road runoff. Consequently, assessing the risk for aquatic environments emerging from TRWP polluted runoff faces the following challenges: (1) Due to structural and chemical variability of TRWP, no standardised sample preparation protocols for (bio-)chemical assessment are available; (2) The lack of environmentally relevant effect data complicates *a priori* decisions on endpoints of interest to investigate. Therefore, comprehensively investigating the ecotoxicity of TRWP demands for a scientific bottom-up approach generating a broad knowledge base covering both chemical and biological effect information for a variety of environmental model scenarios. The project "RoadTox" aims for a quantitative ecotoxicological risk assessment in stormwater runoff sampled from urban and extra-urban roads and federal highways. For this purpose, a comprehensive multi-endpoint bioassay battery paired with hydrological, physical, and chemical characterisation of the received runoff and its particulate matter will be performed. Addressed toxicological endpoints include dioxin-like activity, endocrine disruption, mutagenicity *in vitro*, and sublethal, transcriptional and behavioural effects in *Danio rerio* embryos. The received ecotoxicological information will be integratively analysed and combined with literature data to enable a meaningful risk assessment of the input of tire abrasion into the environment. RoadTox does not solely focus on the individual tire abrasion particles alone but the overall ecotoxicological impact of the road wastewater sample. Based on the quantitative results of the input pathways and the ecotoxicological risk assessment, interdisciplinary recommendations for actions aiming to minimise tire abrasion inputs will be developed. Here we will present the current state of the project, including past and present challenges and first data on physico-chemical profiling, EROD induction, anti-androgenic and estrogenic activity and effects on *D. rerio* embryos. The project is funded by the State Agency for Nature, Environment and Consumer Protection NRW (LANUV).

4.16.T-08 Tire Rubber Microplastics: A Hazardous or Harmless Material?

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Tire rubber may be one of the most popular anthropogenic materials ever produced and consequently, tire rubber microparticles (also called microrubber) are released in very high quantities. In the water environment, tire rubber has been found in concentrations of 0.01 to 155 mg per g sediment dry weight. Furthermore, tire rubber contains many chemical additives that contribute to the material's final characteristics. Additives are potentially released to water and cause concerns related to their toxicity to natural ecosystems. They can be considered as a very complicated chemical mixture that may vary their composition depending on the environmental conditions. In our study, multiple approaches were used to detect the risks of microrubber to freshwater organisms. Factors such as size, sediment type, concentration of microrubber, toxicity of the leachates and three model organisms were used for this purpose. The monitored endpoints were mortality, emergence, sex ration, reproduction and growth (*C. riparius*), reproduction and growth (*Lumbriculus variegatus*) and growth (*Lemna minor*). In addition, gene expression was analyzed in *C. riparius* exposed to low levels of microrubber or their leachates. The results indicated that none of the factors tested related to microrubber particles (size or concentration in sediment) affected any of the endpoints examined in *C. riparius* or *L. variegatus*. In *C. riparius* exposed to leachates, analyses of the dose response curve yielded a LC₅₀ value of 47.3 ± 3.03%, what corresponded to 37.4 g L⁻¹ of tire rubber considering how the leachates were prepared. A similar pattern was found with *L. minor*, showing a reduced growth rate at a concentration of 30%. The gene expression analyses pointed at the generation of oxidative and cellular stress in *C. riparius*. More complete analyses of larvae exposed to particles and leachates separately during 24h and 10d will help us to determine the cellular pathways affected by microrubber and its additives. Our results indicated that organisms likely compensate the initial oxidative and cellular stress and overall are not affected in longer tests. Studies with the leachates indicated a more obvious toxicity that may not be environmentally relevant. However, chemicals present in the leachates may affect other important parameters such as growth at lower concentrations. The results prompted to further investigate the effects of both particles and chemicals present in tire rubber.

4.16.T-09 Evaluation of Tire Tread Particle Toxicity to Fish Using Rainbow Trout Cell Lines

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Tire and road wear particles (TRWP) are generated by the abrasion of tires while driving, and questions were raised recently in regard to their potential contribution to microplastics released into the aquatic environment and their potential toxicological impacts. Our study aimed to determine the toxicity of TRWP and associated chemicals to fish using two Rainbow Trout (*Oncorhynchus mykiss*) cell lines representing the gill (RTgill-W1) and the intestinal (RTgutGC) epithelium. More specifically, we used cryogenically milled tire tread (CMTT) particles as a proxy for TRWP. These were applied to the cells directly, and in the case of RTgutGC cells, also after *in vitro* digestion to investigate if fish gastro-intestinal conditions could result in a different chemical profile and change the toxicity. In accordance with OECD TG249, cell viability was assessed after 24 hours acute exposure using a multiple-endpoint assay indicative of cell metabolic activity, membrane integrity and lysosome integrity.

Chemical composition of the exposure medium was analyzed to assess which chemicals could be responsible for the observed acute effects. We determined EC50 values after direct exposure to RTgill-W1 and RTgutGC cells at 2.01 g/L and 3.80 g/L, respectively, and a maximum no-observed-effect concentration of 10 mg/L. 2-Mercaptobenzothiazole was found at the highest concentration among organic chemicals leaching out from the particles. It could be a major contributor to the loss in cell metabolic activity based on its toxicity to Rainbow Trout *in vivo*, although additional particle or mixture effects cannot be ruled out at this point. Moreover, CMTT digestate EC50 was determined at 12.15 g of tire particle/L of digestive fluid. The observed toxicity could be due to high amounts of Zn, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), 1,3-Diphenylguanidine (DPG) or 2-Mercaptobenzothiazole leaching out during *in vitro* digestion. Although traces of 6PPD-quinone were measured in the exposure medium, further cytotoxicity assays using the RTgill-W1 cell line showed no significant toxicity of this compound up to 3 mg/L. Overall, our *in vitro* toxicity data agree with reports of acute fish (*in vivo*) toxicity and show that acutely toxic concentrations of CMTT are well above concentrations measured in river water (0.3 to 4 mg/L). Because higher concentrations have been detected in road runoff (>100 mg/L), more research into long-term effects of such high exposure is required.

4.16 Tire wear and microrubber particles - from problems to solutions (Virtual Only)

4.16.V-01 Addressing Tire-Related Pollution in Urban Runoff: Mitigation Options and Priority Data Needs

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Recent investigations in the San Francisco Bay Area (California, USA) identified urban runoff as a major pathway for microplastics entering receiving waters, with black rubbery particles from tires estimated to be the single most common microplastic flowing into the Bay annually. Tire particles and associated contaminants pose toxicological risk to aquatic species. For example, one tire-related chemical, 6PPD-quinone (a degradate of a tire antioxidant) causes pre-spawn mortality in coho salmon. Based on conceptual models to synthesize and integrate our current understanding of tire particle sources and pathways to urban runoff, we developed a broad framework of control strategies for tire particles and the water pollutants they carry. The goal of the control strategy framework is to inform future research and management recommendations for managing tire-related water pollution. There are many available approaches to prevent and mitigate tire-related water pollution, from re-formulating tires to remove toxic ingredients, to reducing tire wear rates, to collecting wear debris at the point of emission from vehicles, to downstream collection and treatment of urban runoff to remove tire particles and chemicals. Among the many tire-wear-particle-related data gaps, several were identified as priorities because they have significant implications for future management decisions regarding tire wear debris. These include particle surface area measurements to determine the size fraction of tire wear particles that accounts for the majority of the surface area, which would inform design and location of on-vehicle and environmental systems to collect tire particles. Addressing tire wear particles and associated chemicals will require a holistic approach because it is not possible to separate the tire microplastics from the chemicals they contain, and because the topics that need attention are typically managed by different types of agencies and scientists (air, water, toxic chemicals). Turning research attention toward the priority data needs would inform development of mitigation measures that most cost-effectively address tire wear particle pollution.

4.16.V-02 Concentration of N-(1,3-dimethylbutyl)-n'-Phenyl-P-Phenylenediamine-Quinone (6PPD-Q) in Road Dust and Its Association With Environmental Factors

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone, also referred to as 6PPD-quinone (6PPD-Q), is a by-product of a tire manufacturing additive and was recently identified as a toxic chemical causing acute lethality of coho salmon *Oncorhynchus kisutch* in urban watersheds. Despite its potential occurrence and ecotoxicity in receiving waters worldwide, available information on the occurrence and fate of 6PPD-Q is still limited. We collected dust samples deposited on road surfaces from May to October 2021, and quantified the concentrations of 6PPD and 6PPD-Q in the dust samples. Then we investigated the seasonal trends of the concentrations and association with road types and antecedent dry weather periods on 6PPD-Q concentrations. Our results showed that 6PPD-Q concentrations normalized by organic carbon content were the highest in May and decreased gradually, which may be due to higher atmospheric ozone concentrations in spring. Also, we found that the ratio of 6PPD-Q to 6PPD correlated with antecedent dry weather periods, which is consistent with the fact that the parent chemical, 6PPD, is more easily degraded compared with 6PPD-Q. These results can help to understand the environmental occurrence, fate, and behavior of 6PPD-Q.

4.16.V-03 Evaluation and Method Refinement of Pyrolyzer Technologies for Quantitative Py-Gc-MS of Tire Tread and TRWP in an Environmental Matrix

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Microplastics (MP) are common and widespread particles in the environment that have been detected in a vast array of media, such as marine and drinking water, soil, sediment, food, air, marine mammals, and even humans. Recently, several working groups and regulatory agencies have proposed policies and frameworks for reduction and mitigation of MP pollution in the environment. MP can have a variety of particle morphologies, physico-chemical properties, sizes, colors, and shapes, making their identification and quantification an analytical challenge. Sources of MP include commercial products, textiles, plastics, fishing, and tire and road wear. The abrasion and wear of tires during use produce complex particles defined as tire and road wear particles (TRWP). Analytical methods capable of quantifying the amount of TRWP in the environment are critical to determining the

prevalence and environmental fate, however, the lack of comparisons between alternative mass-based methods represents a critical data need. In support of addressing this information gap, we evaluated three pyrolyzer technologies for pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), Curie point, microfurnace, and resistive, using three unique cryomilled tire tread (CMTT) samples in an artificial sediment matrix following ISO Technical Specification 21296:2017. The tire tread dimer markers used for quantification were 4-vinylcyclohexene (4-VCH), which is a marker for styrene-butadiene rubber (SBR) and butadiene rubber (BR), 4-phenylcyclohexene (4-PCH), which is a marker for SBR, and dipentene (DP), which is a marker for natural rubber (NR) or isoprene. Accuracy and precision for each pyrolyzer were compared for CMTT with and without artificial sediment matrix. Curie point and microfurnace performed well, producing acceptable precision (majority of samples with coefficient of variation < 20 %) and accuracy (majority of samples with CMTT recovery 50 – 200 %), suggesting these pyrolyzers are well-suited for quantitative Py-GC-MS of tire tread and TRWP in complex matrices. Our comparison data supported the subsequent exploration of potential method refinements, such as chromatography parameter modification, chemical pretreatment, and thermal desorption using microfurnace Py-GC-MS. Overall, these method refinements improved peak resolution and minimized interferences, supporting the utility and applicability of Py-GC-MS for quantification of TRWP and MP in complex environmental samples.

4.16.V-04 Unraveling the Toxicity of Tire-Wear Particle Contamination in Aquatic Organisms: From Chemical Mixture to Nanoparticle Toxicity

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From atmospheric urban pollution to urban runoffs, tire-wear particles are a concerning source of pollution and their ubiquity is raising global awareness. Indeed, the potential impacts of tire-wear particle pollution in aquatic ecosystems are of concern. Effects have been investigated in multiple species, from single-celled algae species to fish and amphibians, highlighting a potential harmful impact on several levels of the trophic chain. This raises questions regarding the effects on populations and ecosystems dynamics, and also regarding the mode of action of this complex mixture. Our work aimed to shed light on the toxicity of the different components of tire wear leachate, i.e. the chemicals vs nanoparticle fractions, on four freshwater model species of different trophic levels: *Chlorella vulgaris*, *Lemna minor*, *Daphnia magna*, and *Silurana tropicalis*. Through a series of filtrations, tire-wear particle leachate was divided into three different fractions: one fraction regrouping all components, i.e. dissolved organic chemicals and heavy metals, as well as particles below 0.2 µm, the second fraction containing only the dissolved species (i.e., heavy metals and organic chemicals), and a third fraction composed only of particles below 0.2 µm. The acute toxicity of each fraction was assessed in single-species assays using morphological endpoints, immobility and mortality, using modified standard OECD protocols. Our results showed that our separation method was effective in separating tire wear dissolved chemicals and particles, and that overall toxicity based on morphological endpoints and EC50s were different from one fraction to another, and among species. Some species showed high mortality at low concentrations, such as *D. magna*, whereas photosynthetic organisms required much higher concentrations to observe an effect on morphological endpoints and a decrease in growth. Overall, the whole fraction containing dissolved chemicals and particles was more toxic than the separated chemical and particulate fractions, suggesting a synergistic or additive effect between particles and chemicals. The overall sublethal effects observed during acute exposures are of concern in a context of potential chronic exposure, such as urban runoffs and atmospheric deposition, and raise questions regarding the potential impact of this pollution on trophic chains and population dynamics.

4.16 Tire wear and microrubber particles - from problems to solutions (Poster)

4.16.P-Mo230 Can We Trust Current Tire Wear Particle Emission Estimations?

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Tire wear particle (TWP) emissions gained recently more attention since they are considered to contribute a major share (estimates are up to 95%) to the overall microplastic emissions. Furthermore, TWP are suspected to be harmful to flora, fauna and humans. To estimate TWP emissions, recent studies calculated country-based emissions based on emission factors or material flow analysis. Here, we provide a closer look at the quality of 13 country based TWP emission studies (for Europe) published since 2000. We searched for all references and references of references which were used by these studies to derive TWP emissions by using internet search engines, libraries and e-mail contacts. We categorized all publications and arranged them in a network of publications showing who references whom and where the studies originally derived their data from. We found that the TWP emission studies base their TWP related information on a wide network of 66 publications. However, only in very few cases (15%) the studies directly referenced to a publication that actually measured TWP emissions. Mostly a study referenced to a review which either summarized different (review) studies or just referenced to another study. In total, we found only eight studies which experimentally measured TWP emissions but four of these studies originate from the 1970s, one analysed light vehicles and one only considered busses. This leaves us with only two about 20-years-old non-peer reviewed reports as basic source of release of TWP from tires during driving. Additionally, both of these studies are not referenced more than three times in the publication network. Considering how important TWP emissions are as a pollution in the environment, we were surprised how few studies with actual measurements we found and how many reviews are referenced overall. This clearly shows that the current TWP emission estimates are subject to high uncertainties which should be clearly communicated. We argue that new studies are

urgently needed, especially considering new developments in the car industry (e.g. heavier cars like SUV or e-mobility) or in the tire industry. We assume that policy makers would be highly interested in accurate TWP emission data to estimate potential risks with more confidence. Also, successfully implementing measures to reduce TWP emissions to the environment relies on such data.

4.16.P-Mo231 Effects of Tire Particles on the Reproduction of Earthworms (*Eisenia fetida*) and Bioaccumulation Potential of Tire-Related Chemicals

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Tire and Road Wear Particles (TRWP) are produced during the wear of tire rubber on the road pavement and contain various chemicals originating from the road environment or from the rubber itself. Field measurements suggest that concentrations of TRWP could reach 0.1 - 100 g kg⁻¹ on the road side and the soil adjacent to the road. However, potential toxic effects of these particles on the soil organisms remain under investigated. Therefore, this study investigated the effects on survival, growth and reproductive output of environmentally relevant tire particle concentrations in soil on the earthworm species *Eisenia fetida*. The bioaccumulation potential of several commonly used tire-related chemicals was established and their bioaccumulation kinetics were determined. Cryogenically Milled Tire Tread (CMTT) particles were used as a surrogate for environmental TRWP. Four treatments were tested: Control soil (no CMTT), low contaminated soil (0.05% CMTT), high contaminated soil (5% CMTT) and a soil contaminated with CMTT leachate only (100 gCMTT/L). The tissue concentrations of 4 Polycyclic Aromatic Hydrocarbons and 5 other tire-related chemicals in the earthworms exposed to the treatment with CMTT leachate only, or to 0.05% CMTT, did not differ from the control. This was different for the worms exposed to 5% CMTT which exhibited much higher tissue chemical concentrations than the control. The Biota-Soil Accumulation Factor (BSAF) determined for the organisms exposed to the high contaminated soil were < 1, except for the apolar phenanthrene, fluoranthene and pyrene where the BSAF ranged from 1 to 2. This indicates that the tire-related chemicals exhibit a low bioaccumulation potential in *Eisenia Fetida*. A slight increase of the reproductive output with increasing CMTT concentration and a slight decrease of the weight of the juveniles exposed to CMTT was observed in the reproduction test. A possible explanation is that the higher energy cost for adult organisms to increase reproduction rate could lead to the lower individual weight of the juveniles observed. An alternative hypothesis is that the cocoons or juveniles were affected by the exposure to CMTT which prevented normal development or growth compared to the control treatment. More research is needed to confirm these results and reveal the underlying physiological mechanisms involved.

4.16.P-Mo232 Studies on Microrubber and Tire Accumulation Along Roadsides of Al Ain, Eastern Region of Abu Dhabi Emirates, UAE

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Materials made of rubber are a source of polymers released into the environment, where tire abrasions are a major contributor. In many hot, arid environs, whole tire losses are more common than in more moderate climates, and this can lead to the accumulation of additional tire material on the road sides, which over time can be the source of secondary microtires. Due to the lack of precipitation it is expected that modes of microtire relocation are more limited than in more moderate climates. This 2-year study examines the rubber/tire content from 23 sampling sites along roads within the city of Al Ain, Eastern Abu Dhabi Emirate, United Arab Emirates. The samples represent soils along roadsides and road dust, specifically from road curbs. Over 100 days, the fate of a number of sequestered rubber/tire particles was investigated, both in their natural locations on the road and along the roadside as well as embedded in soil under controlled outdoor laboratory conditions. Samples showed 2 – 148 microtires per g soil and 14-119 microtires per g road dust. It has been found that rubber/tire materials desiccate over time in the high temperatures found in the summer, become brittle and can break into much smaller pieces, resulting in micro-tire particles. Study of run-offs showed that tire particles are swept into storm drains during larger, very infrequently occurring rain events. However, the predominant mode of transport was found to be through air movement. Nevertheless, samples taken from an artificial lake and one Wadi (creek bed that seasonally carries water) within the confines of Al Ain, but off-road, have shown only few microtire particles so that the dispersion of microtires is not as pervasive as had been expected.

4.16.P-Mo233 Tyre Wear Particles and Small Microplastics (SMPs <100 Mm) From Highway Stormwater Runoff: Sampling, Pre-Treatment Procedures and First Results

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Highway stormwater runoff is a direct pathway for conveying microplastics (MPs) and other environmental pollutants from land-based sources to the aquatic and terrestrial environment. However, the concerns on stormwater for microplastic pollution and the other components of microlitter (additives and plasticizers) have grown exponentially and the knowledge on their pathways and impacts are now very limited. Important sources of microplastic in stormwater runoff are related to traffic vehicles, polymer-modified bitumen, road paint, atmospheric deposition and the wear of tyres. Hence, due to their small size, smaller MPs (SMPs, < 100 µm), additives and tyre wear particles (TWPs) can pose a risk for the environments as they can be ingested by different kinds of biota and can cause potential health problems for human beings. Only very limited studies have been paid attention to microplastics, especially SMPs, and TWPs from highway stormwater runoff with a consequent lack of knowledge regarding the

amounts, size, and composition of these pollutants emitted in the different environmental pathways. There are currently no standardized methods and harmonized techniques for the collection, pretreatments, contamination procedures, and analysis of SMPs and TWPs from stormwater runoff, making significant differences in the data of these pollutants studies difficult to compare. In this study, stormwater runoff samples were collected during different rainfall events from a trafficked highway near Venice, Italy. A pre-treatment procedure (e.g., oleo-extraction, purification, and filtration) was developed and optimized in a Clean Room (ISO 7) at Ca' Foscari University of Venice to prevent any possible plastic contamination. Quantification and simultaneous chemical identification were performed via MicroFTIR for SMPs, TWPs, additives, and plasticizers in stormwater runoff samples. Further, pyrolysis gas chromatography-mass spectrometry (Pyr-GC/MS) was developed to confirm the presence of specific SMPs and TWPs in the same samples analyzed. The cross-validation among MicroFTIR and Pyr-GC/MS techniques allows the standardization of pretreatment and analytical methods. Regarding TWPs, different tests were carried out and the first results of quantification and chemical identification of specific markers were obtained.

4.16.P-Mo234 Model Particles for Tire Wear Fate Studies

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An increasing number of studies investigate the organic constituents of tire and road wear particles (TRWPs), their transport into the environment and their potential effects on biota. Cryogenically milled tire tread (CMTT) is available as a relatively affordable and available testing material. However, the extent to which CMTT as test material compares with TRWPs has not been fully evaluated. This knowledge is important for a transfer of lab results with CMTT to the environment. Using a range of analytical techniques, this study compares the physical and chemical properties of CMTT with those of TRWPs that were generated using a tire test bench mimicking driving conditions to produce particles. Properties of this TRWP material are expected to be similar to those generated during normal driving. Physical and chemical properties of samples of CMTT and TRWP were investigated by a number of analysis techniques. Electron microscopy, particle size distribution, organic constituents (extractables) and the release of organics in water (leachables) are presented. This study shows marked differences of CMTT compared to TRWPs. CMTT particles have a different shape and a larger mean particle size compared to TRWPs. These differences in size and shape between CMTT and TRWPs may affect the release of organic constituents from the material. CMTT exhibited much higher contents of functional chemicals (factor 4 – 40), such as vulcanization accelerators and antioxidants. Reactive organics are transformed during the use of tires and the abrasion process due to heat and oxidation. The different reservoirs of organics in the specimen of CMTT and TRWPs led to higher concentrations in the leachate of CMTT. However, leaching appeared to be modulated by physical properties of the particles under study. Although CMTT is a more readily available test material, it may not always be a suitable surrogate for TRWPs. Its differences to TRWPs should be considered closely when interpreting lab results generated from CMTT.

4.16.P-Mo235 Flow Conditions and Retention of Road Related Microplastics in a Gully Pot: A Numerical Study

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Tyre and road wear particles (TRWP) are reported to be one of the largest sources of microplastics in the environment. TRWP are transported to surface waters and oceans by road runoff through stormwater systems. The presence of TRWP has been reported in sediment samples from gully pots. Gully pots are the first-line treatment step in the urban stormwater system, meaning that they can improve stormwater quality if TRWP can settle in the sand trap. However, there is also a risk that the gully pot can release TRWP during heavy rainfalls if larger flows can mobilize TRWP previously settled. Earlier studies on the efficiency of gully pots at retaining sand and other particles point to the synergetic importance of several factors: discharge flow rates, gully pot geometry, sand trap depth, amount of air bubbles entrained, and particle properties. These factors can be simultaneously evaluated using a modelling approach that can resolve details of the flow and take into consideration the geometry of the gully pot and the particle characteristics. In our study, computational fluid dynamics (CFD) was used to simulate the flow of water and air in a gully pot. A three-dimensional model of a gully pot, 0.35 m wide and with a sand trap depth of 0.39 m (full depth 1 m), was set up using commercial CFD software Star-CCM+ version 20.3. The volume of fluid method was used to describe the water and air phases. The main findings from the CFD model were validated against experimental data. The simulations indicated that air interaction largely affects the water flow, and that discharge volume causes different flow features in the gully pot. By gaining detailed knowledge of the hydraulics in the gully pot, specific flow features are visualized and the residence time of the water is determined in order to predict the effect on the settling of TRWP. Additionally, the CFD model will be coupled to Lagrangian particle tracking to simulate the behaviour and fate of individual particles in the gully pot. In this way, the retention of TRWP and the effects of size and density of TRWP on settling can be evaluated. This type of detailed simulations can be implemented in large scale models to describe transport processes affecting the fate of TRWP in the stormwater system. Modelling can hence be a useful tool to predict the occurrence of TRWP in stormwater systems and in the environment, to evaluate remediations techniques, and to support decision-making.

4.16.P-Mo236 Evaluation of Manufactured Treatment Devices in a Separated Stormsewer to Capture Low- and High-Density Tire Road Wear Particles

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Tire road wear particles (TRWPs) emitted by vehicles due to the friction of tires on road surfaces are increasingly being recognized as a dominant fraction of microplastic/rubber in traffic-affected areas. TRWPs vary in density, size, shape, and

composition. Tire material has long been a subject of ecotoxicity assessment due to observations of toxic leachates. Due to potential toxicity, preventing environmental emissions of tire microrubber should be a priority. It is known that TRWPs are abundant in roadside soils and stormwater runoff and may accumulate in rain gardens and stormwater ponds, however, research is limited on fate of TRWPs in grey-stormwater infrastructure. Therefore, we investigated TRWP capture effectiveness of stormsewer inlet devices, also called manufactured treatment devices (MTDs). Sediment samples were taken under dry conditions from the chambers of 7 MTDs in Mount Pleasant, South Carolina USA. MTDs came in two varieties: a multi-chambered, flow-through baffle design and a single-chambered hydrodynamic vortex separator. Samples of road dust and sediment around the discharge site were also collected. Sediments were dried and sieved and analyzed for grain size, organic matter, and heavy metal content. Density separations were performed on the target sieve fraction (63-500 micron) using a NaCl solution (1.2 g/mL) and then a sodium polytungstate (1.9 g/mL) to distinguish high- and low-density fractions. TRWPs in these fractions were identified by morphology and resistance to heat, counted, and size measured using stereomicroscopy. A subset of suspected TRWPs were taken for pyrolysis-GCMS and scanning electron microscopy for quality assurance. In a preliminary analysis from 2 study sites, the concentration of low-density (< 1.2g/mL) and high density (1.2-1.9 g/mL) TRWPs across samples ranged 80-300 particles/g dry wt. and 150-400 particles/g dry wt., respectively. Although still under investigation, early results show that TRWP concentrations are ~2x higher in MTD sediments than in road dust. We will further evaluate if the size/shape and density of TRWP in the micro-size change across the stormwater pathway from road source through MTDs to environmental discharge points in tidal creeks. Analysis of the quantity and character of TRWPs as well as relationship to other sediment characteristics at our study sites will inform on the ability of advanced stormsewer infrastructure to mitigate roadway stormwater runoff sources of TRWP pollution.

4.16.P-Mo237 Ingestion of Crumb Rubber and Body Burden of Associated Contaminants in Marine Invertebrates and Fish

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Crumb rubber granulate (CRG) produced from end of life vehicle tires is widely used on artificial sports fields and to create urban artificial surfaces. It is known to contain a wide range of organic chemicals and metals, some of which are only just being identified and assessed. Weathering of such artificial surfaces releases both particles and the chemicals they contain into the environment, where they may be bioavailable and affect biota. *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine (6PPD) has been identified as a chemical marker for CRG and tire wear particles. Both 6PPD and its transformation product, 6PPD-quinone, have been shown to be toxic to a number of different freshwater and marine organisms. The current study examines the ingestion of CRG (1 - 2.8; < - 1.2 mm) by a marine invertebrate and fish, the gut retention time of ingested CRG, and the body burden of tire-derived chemicals. Lumpfish (*Cyclopterus lumpus*) and Northern shrimp (*Pandalus borealis*) were exposed to CRG in laboratory experiments for 21 days; 7 days of exposure to CRG followed by 14 days of depuration. The stomach contents have been analyzed for ingested CRG and selected tissues (Lumpfish liver and blood; Northern shrimp hepatopancreas and muscle) have been characterized for tire-associated chemicals and metals. Analytical chemical techniques involved ICP-MS, pyrolysis GC-MS, GC-MS/MS and HRGC-HRMS (ThermoFisher, Orbitrap). CRG were found in 76% of exposed Lumpfish stomachs (n=90), where the number of CRG particles in the stomach increased during the exposure and peaked around day 8 followed by a decrease throughout the depuration. Ingested CRG was still found in some Lumpfish stomachs at the end of the experiment (day 21). Ingestion by Northern shrimp have been determined by pyrolysis-GC/MS. Analysis by HRGC/HRMS (ThermoFisher, Orbitrap) detected 6PPD (0.1 – 0.8 ng/μL) in 19% of Lumpfish blood samples analyzed (n=36), both at the end of the exposure and at the end of depuration. ICP-MS analysis of metals did not show increased concentrations of metals in any of the tissues for exposed individuals or over time for either Lumpfish or Northern shrimp. The current results suggest that ingested CRG can stay in the digestive system of Lumpfish longer than 14 days and that the particles may be leaching 6PPD during this period, suggesting bioavailability of CRG particles and associated chemicals to ecologically and commercially important marine organisms.

4.16.P-Mo238 Investigation on Toxic Effects of Diversely Polluted Road Effluents on Fish Embryos Using *Danio rerio* As a Model Species

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While the number of vehicles registered worldwide is increasing, the fate of their remains continues to gain importance and presents a global environmental issue. Despite the large quantity of vehicle generated debris and the associated toxicological risk to the environment, the toxic effects of tire wear particles (microplastics), vehicle fluids, and other vehicle debris have been barely studied in environmental toxicology. The effects on the aquatic environment were addressed in the present study, which was created as part of the project 'Ecotoxicological assessment of tire abrasion in stormwater runoff of heavily trafficked roads – RoadTox' at the Department of Evolutionary Ecology and Environmental (E3T), Goethe University, Frankfurt am Main in cooperation with the Institute of environmental engineering (ISA), RWTH Aachen University. The road effluent samples have been taken from urban and extra-urban roads and federal highways in North Rhine-Westphalia (NRW), Germany. The overall toxicity of the samples on the aquatic environment was investigated, aiming for an ecotoxicological risk assessment. Road

effluents are chemically complex environmental samples composed of various vehicle debris rather than tire particle abrasion alone. Therefore, both filtered and unfiltered native road effluent samples were used instead of samples of individual substances or enriched extracts. This study used *Danio rerio* (zebrafish) as a well-established freshwater fish model species up to 120 hours post-fertilization. The fish embryo acute toxicity test (FET DIN EN ISO 15088) was performed for determining the acute (sub)lethal toxicity as well as the swimming behaviour, different *in vivo* biomarkers (e.g., acetylcholine esterase, EROD activity) and heartbeat tracking for cardiovascular abnormalities. These studies with *D. rerio* are part of the RoadTox project's comprehensive bioassay battery and consisted of several assays examining multiple sublethal endpoints (presented separately). The project is funded by the State Agency for Nature, Environment and Consumer Protection NRW (LANUV).

4.16.P-Mo239 Genotoxic and Estrogenic Effects of Cryo-Milled Tire Tread Particles Detected With High Performance Thin-Layer Chromatography (HPTLC) Based Bioassays

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Vehicle tires abrade during use, generating particles that can be transported to aquatic environments. This work investigated (1) the worst case estrogenic and genotoxic potential of chemicals in tire particles, and (2) a more realistic exposure scenario of tire particle ingestion by fish. For (1), organic chemicals were comprehensively extracted from cryogenically milled tire tread (CMTT) with Soxhlet extraction. In (2), we approximated a fish digestion scenario with successive incubation of CMTT particles in simulated gastric and intestinal fluids. In these samples, we first identified and quantified eleven organic chemicals suspected of being associated with tire wear particles with LC-MS/MS. We evaluated estrogenicity and genotoxicity of the individual chemicals and CMTT samples with a yeast estrogen screen (YES) and umuC Chromotest (umuC) without metabolic activation, respectively, directly on HPTLC plates after chromatographic separation. Two individual chemicals were active in HPTLC-YES: N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and 2,2'-dithiobisbenzothiazole. Three chemicals were active in HPTLC-umuC: N-cyclohexyl-2-benzothiazolesulfenamide, cyclohexylamine, and 1,3-diphenylguanidine (DPG). Through chromatography, multiple estrogenic and genotoxic signals were detected in (1), total CMTT extracts, with up to 1 mg CMTT applied to HPTLC plates. Among the eleven chemicals measured in CMTT, DPG is one that was likely responsible for umuC genotoxicity of CMTT extracts. It was measured in CMTT at levels above the biological detection limit of umuC (2.5 µg in CMTT extract vs 0.65 µg lowest effect level, after applying to HPTLC plates), and the individual compound shared the chromatographic position of genotoxicity observed in CMTT extracts. In (2), simulated digestates of CMTT did not induce a response in either HPTLC-YES or HPTLC-umuC assay. Accordingly, DPG was also measured in CMTT digestates, although below the lowest effect level of DPG alone (0.27 µg in CMTT digestate vs. 0.65 µg lowest effect level). In summary, genotoxic and estrogenic hazards were (1) detected in comprehensively extracted tire particles, but were (2) below biological detection in simulated digestates, suggesting low concern in such a scenario. Future efforts will work to identify the remaining unknown toxicants to allow risk evaluation of these hazards and determine effects of environmentally representative tire and road wear particles.

4.16.P-Mo240 Plant Uptake of Tire Derived Chemicals

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Tire wear particles, which have gained attention in recent years due to the toxicity of additives that leach from them, are ubiquitously present in urban stormwater. Many cities combine stormwater with municipal wastewater and treat it in conventional wastewater treatment plants, where, like thermo-microplastics, tire wear particles are expected to settle to sludge. Wastewater sludge, containing tire wear particles, is then often applied to agricultural fields as fertilizer. We expect that tire wear particles leach most of their additives once on the field, due to their relatively short residence times in WWTPs, and their comparatively slow additive release rates. This could pose a serious health risk to humans if edible plants take up and accumulate these additives from soil, a phenomenon which is well established for other classes of organic micropollutants. Therefore, in this study we evaluate lettuce uptake of 5 commonly detected tire additives: diphenylguanidine (DPG), hexa(methoxymethyl)melamine (HMMM), benzothiazole, N(1,3-dimethyl-butyl)-N'-phenyl-p-phenylenediamine (6PPD), and 6PPD-quinone. Under hydroponic conditions, we expose lettuce in one scenario directly to additives, and in another scenario, to tire particles themselves. We monitor the resulting concentrations of additives in lettuce roots and leaves, as well as in hydroponic nutrient solution, using a modified QuEChERS extraction method followed by measurement via liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS). After exposure, we remove lettuce plants to clean nutrient solution, and monitor degradation of the compounds in the plants over time. We also measure sorption of additives to root material, since high affinity to root material can impede translocation to leaves, which limits the amount of compounds reaching the edible part of the plant. Preliminary results indicate that all 5 additives are taken up by lettuce roots and are translocated to leaves. On the other hand, initial experiments indicate that stability in leaves is compound dependent. When looking at uptake results from a mass balance perspective, we find that less than 0.01% of compounds added to nutrient solution are taken up by plants, leading us to assume that except under very high concentrations, tire wear particles in agricultural sludge do not pose a serious health risk to humans.

4.16.P-Mo241 Reducing the Environmental Impact of Tire Wear Using a Supply Chain Approach

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Globally, tire wear is responsible for approximately 50% of the total amount of microplastics released to the environment. It is therefore not surprising that this topic has received an increasing amount of interest, both inside and outside the scientific world. In order to effectively reduce the environmental impact of microplastic particles, it is essential to understand the root causes and the pathways of microplastic particles in the environment. This study presents an approach that can support the identification and prioritization of mitigation measures for reducing microplastic pollution caused by tires. The approach links the quantification and spatial modelling of microplastic emissions to particular human activities using a supply chain approach. As a starting point, the tire supply chain, including resource extraction, production, use and end-of-life, is mapped to provide insight in the main leakage points and to provide a framework for close collaboration with stakeholders. Information for the supply chain mapping is derived from literature and stakeholders. As a mapping method SCOR is used, which is a process reference model for supply chain management. SCOR includes the mapping of processes, activities and performance, but puts limited emphasis on environmental impact. Subsequently, the main leakage points are described in terms of their contribution to microplastic emissions and environmental impacts. The emissions are quantified using an LCA-based approach, which results in an aligned functional unit to describe the quantified microplastic emissions from tire wear for the different supply chain links. The ultimate aim is to apply this approach in a specific geographical area and to explore the impact of different alternative interventions aimed at minimizing tire wear emissions.

4.17 Toxicokinetics and toxicodynamics in chemical risk assessment: data collection and model development

4.17.T-01 Accumulation-Depuration Data Collection in Support of Toxicokinetic Modelling

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Environmental Risk Assessment (ERA) for chemical substances often requires a bioaccumulation test which consists in both an accumulation and a depuration phases. The internal concentration over time within organisms is regularly measured during both phases. Output data are then analysed by a toxicokinetic (TK) model with uptake and elimination parameters to be estimated and used to provide bioaccumulation metrics as dimensionless ratios (e.g. BCF/BSAF/BMF). TK models are chosen according to input data; accounting for several exposure routes and different elimination processes, including metabolism. Regulatory speaking, bioaccumulation metrics are compared to decision thresholds to classify chemical substances as non-, low-, mid- or very bioaccumulative. MOSAIC_{bioacc} is a web application freely available from <https://mosaic.univ-lyon1.fr/bioacc>. It allows easy calculations of bioaccumulation metrics in an automated way. This avoids stakeholders investing in underlying technicalities, while benefiting from the most advanced modelling and inference methods, recently developed to improve effectiveness and robustness of bioaccumulation outcomes in full respect of regulatory needs. In particular, MOSAIC_{bioacc} systematically provides uncertainties on outcomes. It can deal with for any kind of species-compound combinations. Based on daily usage statistics, we know that MOSAIC_{bioacc} users are either from academia, industry or regulatory bodies, and from a lot of countries worldwide. The huge lack of raw TK data, together with an increasingly private collection of data sets got from the scientific literature, led us to the conclusion that a new publicly available accumulation-depuration (TK) database became essential, in full respect of the Findable, Accessible, Interoperable and Reproducible (FAIR) data principles. To date, our TK database gathers together more than 210 data sets, for more than 50 genus and more than 120 chemical substances. In this talk we will present a full meta-analysis of these data sets that are all available in a standardised format directly usable in MOSAIC_{bioacc}. Hence, we used the same modelling approach in calculating bioaccumulation metrics, a necessary condition to compare potential of chemically similar substances to bioaccumulate in a given species, for example. Our purpose will also be to illustrate the really added value of sharing data in the era we recently entered with Open Science.

4.17.T-02 A Generalised Physiologically Based Kinetic Model for Fish for Environmental Risk Assessment of Pharmaceuticals

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With increasing medical and veterinary use, pharmaceuticals have been detected as emerging pollutants in global water bodies. Many of these pharmaceuticals reside in aquatic organisms such as fish, potentially imposing adverse effects on organism survival or fitness. Physiologically based kinetic (PBK) models have become essential risk assessment tools since they allow a mechanistic framework to understand toxicological effects in organisms. Fish is often selected as a model organism. However, fish PBK models often focus on specific species, which is impractical in a risk assessment context given the countless species. Moreover, many pharmaceuticals are ionisable and fish PBK models accounting for ionisation are rare. Therefore, the present study aimed to develop a generalised PBK model applicable to various fish and ionisable chemicals and to assess its performance. Fish organ weights, cardiac output and oxygen consumption were estimated through allometric scaling, and the Boltzmann-Arrhenius equation was applied to adjust for temperature differences. Exchange and partition coefficients were estimated through existing quantitative structure-activity relationship (QSAR) models, taking pH influence into account. Model performance was subsequently evaluated for five pharmaceuticals covering neutral and ionic structures. Consequently, only the fish body mass, the chemical properties and the exposure scenario were required as input. Two sets of results were computed. With biotransformation half-lives (HL) from EPI Suite, 73% and 41% of the estimations were within a ten-fold and a three-fold difference from measurements, respectively. With experimental biotransformation HLs, the performance improved to 87% and 59%, respectively.

For ionisable substances, only 24% of estimations were outside a ten-fold, more accurate than any of the existing species-specific PBK models. The present study is the first to develop a generalised fish PBK model that focuses on mechanism-based parameterisation and explicitly accounts for ionisation. The generalised model would facilitate its application across chemicals and species, improving efficiency and feasibility in a risk assessment context and supporting an animal-free toxicity testing paradigm. Given the evidence of accuracy and a lack of generalised PBK models to date, we regard our work as an essential step forward in chemical risk assessment.

4.17.T-03 Evaluating Predictive Toxicokinetic Models in *Daphnia magna* to Support Environmental Safety Decisions

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The large number of chemicals in use today represents a major challenge for regulators worldwide in determining environmental safety thresholds for chemicals whilst minimising the generation of new toxicity data. One proposed strategy for advancing the pace of chemical risk assessment is leveraging the use of *in vitro* data by creating robust quantitative models that predict the exposure levels of chemicals in environmentally relevant species. Toxicokinetic modelling is a new approach methodology (NAM) that provides reliable predictive capabilities which could lead to reduced animal use and increase efficiency, in terms of time and cost. While toxicokinetic models have been developed in some environmentally relevant species such as fish, these are still limited in invertebrates. Current available toxicokinetic models for *Daphnia magna* are chemical specific, derived by fitting individual chemical data, with limited application to other chemicals. This study aims to evaluate a range of one-compartment toxicokinetic models to predict the uptake and elimination of a suite of chemicals in *D. magna* by evaluating the models in terms of their accuracies of maximum internal concentration - C_{max} and the chemical uptake and elimination dynamics. Additionally, we plan on using *in silico* predictions of log K_{ow} and log D_{ow} to see whether these toxicokinetic models retain their performance when using predicted values to replace experimental values. To do so, a code infrastructure that encompasses multiple predictive uptake and partitioning models was developed in Python. All model combinations were found to predict C_{max} consistently when comparing predicted and experimental C_{max}, with the best and worst model combinations resulting in 90.4% and 67.3% of the predicted C_{max} values lying within 10-fold of the experimental C_{max}, respectively. However, preliminary results on the use of these models to simulate the uptake and elimination dynamics show significant deviation between predictions and experimental data, suggesting further work on model adjustment is needed. Additionally, our work has shown the potential to use predicted values to replace experimental log K_{ow} values without significant impacts on model performance, which furthers its applicability. This work could significantly improve regulatory and safety decision applications specifically in chemical risk assessment by providing robust predictions for C_{max} thus allowing for the use of *in vitro* data.

4.17.T-04 An Amended In Vitro-In Vivo Extrapolation Model to Consider First Pass Clearance Effects on Chemical Accumulation in Fish

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In vitro-in vivo extrapolation (IVIVE) approaches have proven useful for evaluating the impact of biotransformation on chemical bioaccumulation in fish. Most IVIVE efforts to date have focused on predicting chemical bioconcentration in fish (aqueous only exposure), with less attention paid to the effect of biotransformation on chemical accumulation via the diet. Following dietary exposure, biotransformation of a chemical in the gastrointestinal tract (GIT) lumen, GIT epithelia, and liver can greatly reduce its accumulation. However current IVIVE models do not consider first pass clearance effects on dietary uptake and generally only predict biotransformation impacts on whole body elimination. Failure to model these processes correctly could result in underestimation of the true impact of biotransformation on chemical accumulation. Here we present an amended IVIVE (a-IVIVE) model that accounts for the effect of first pass clearance on chemical bioaccumulation in fish. The a-IVIVE model was parameterized using hypothetical data from typical *in vitro* biotransformation assays. We then simulated how biotransformation in the liver and GIT epithelia (alone or combined) may impact chemical accumulation occurring following dietary exposures. First pass clearance can greatly reduce the dietary uptake of contaminants; however, these effects are only apparent at rapid rates of biotransformation (i.e., for intermediate to high extraction efficiency chemicals). These first pass clearance effects were more pronounced when biotransformation in the GIT epithelia was considered, due to the combined impact of biotransformation in both tissues. Empirical *in vitro* biotransformation data and *in vivo* dietary uptake data for two PAHs (pyrene and benzo[a]pyrene) and two sunscreen agents (EHMC and octocrylene) were used to evaluate the relative contribution of the liver and GIT in mitigating dietary uptake and accumulation. For most chemicals, biotransformation in the liver and GIT epithelia could not explain reduced dietary uptake efficiencies reported *in vivo*. One possible explanation for this discrepancy is degradation occurring in the GIT lumen. These findings are consistent with previous work which suggest that luminal biotransformation can greatly reduce the dietary uptake of some organic contaminants. Focused studies investigating luminal biotransformation in fish should be conducted to corroborate these modelled results.

4.17.T-05 Organotropism, Bioaccumulation and Fate of Cadmium (Cd) and Zinc (Zn) in *Gammarus fossarum*, After Exposure to Environmentally Relevant Concentrations

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To improve the toxicity assessment on aquatic organisms, studying their organotropism through a dynamic approach can help to identify the tissues/organs playing a key role in metal regulation (e.g. storage or excretion) and therefore those of interest in ecotoxicology and biomonitoring. This study aims to compare for the gammarid *G. fossarum*: i) the organotropism between a non-essential, Cd, and an essential metal, Zn, at environmentally relevant concentrations; ii) the relative contribution of each organ in the total body burden; and iii) the contrasting internal regulation of both metals. Gammarids were exposed for 7 days (uptake phase) in ^{109}Cd and ^{65}Zn radiolabelled water at $52 \text{ ng}\cdot\text{L}^{-1}$ and $416 \text{ ng}\cdot\text{L}^{-1}$, respectively, and then placed in clean water during 21 days (depuration phase). At several sampling times, the target organs (i.e. caeca, cephalon, intestine, gills, and remaining tissues) were recovered and the Cd and Zn contents quantified by gamma-spectrometry. A one-compartment TK model was fitted, by Bayesian inference, on each organ/metal data set, leading to an estimation of TK parameters. The results show: i) a contrasting distribution pattern of concentrations at the end of the accumulation phase (7th day): gills > caeca > intestines > cephalons > remaining tissues for ^{109}Cd and intestines > caeca > gills > cephalons > remaining tissues for ^{65}Zn ; ii) Cd is slowly lost by all organs, especially by the gills where no loss was measured after a 21-day depuration phase; whereas Zn decreases sharply in all organs after 24 hours in the depuration phase; iii) the uptake and elimination rates were significantly lower for Cd than for Zn, except for the accumulation rate of gills; and iv) surprisingly, under conditions of dissolved contamination, the intestine plays a central role in the accumulation and removal of Cd and Zn, and to a greater extent than for the gills in the case of Zn. One of the major outputs of this work is the preponderant role of intestines in the uptake of metals under conditions of dissolved contamination, whereas the literature tends to show that the gills are the main pathway in freshwater crustaceans.

4.17 Toxicokinetics and toxicodynamics in chemical risk assessment: data collection and model development (Poster)

4.17.P-Tu278 Pharmacometrics Versus Toxicometrics: (Dys)Similarities?

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Whether one develops work in the field of pharmacology or in the field of toxicology, one can identify many points of both convergence and discrepancies. This is what this presentation proposes to highlight by identifying in these two major research areas what can be considered as similar or not in terms of questioning, hypotheses, mechanisms, experimental considerations, as well as modelling approaches. We will especially focus on quantitative approaches in both fields leading to either pharmacometrics or toxicometrics, that is methods in support of decision making either by health care personnel in the field of pharmacology, or by regulatory agencies in risk assessment in the field of environmental toxicology. Our comparison of quantitative approaches in pharmacology and toxicology will be based on two concrete examples. The first one comes from pharmacology and more specifically from the use of pharmacometrics on anti-infective agents employed in treatment of cystic fibrosis to better determine the right dose of antibiotics to use accounting for the between-individual variability. The second one comes from ecotoxicology where toxicometrics are used to assess environmental risks on freshwater invertebrates when they are exposed to heavy metals accounting for internal contamination routes. For both examples, kinetics modelling frameworks as recently developed will be presented and compared in their (dys)similarities.

4.17.P-Tu279 Models Used to Predict Chemical Bioaccumulation in Fish From In Vitro Biotransformation Rates Require Accurate Estimates of Blood-Water Partitioning, and by Extension Chemical Volume of Distribution

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In vitro-in vivo extrapolation (IVIVE) approaches have proven useful for evaluating the impact of biotransformation on chemical bioaccumulation in fish. For the extrapolation of biotransformation data, the sorptive capacity of the *in vitro* test system (e.g., liver S9), blood plasma, and the whole organism need to be considered. Liver S9-water (P_{S9W}) and blood-water (P_{BW}) partition coefficients are used to estimate a hepatic clearance binding term (f_U) that accounts for differences in bioavailability in either system. Several authors have reported that modelled bioconcentration factors (BCF) are sensitive to the selected f_U value, which may contribute to discrepancies in IVIVE modelling efforts. However, more recent research indicates that f_U remains relatively constant for substances with a $\log K_{OW} > 4$. Empirically derived or composition-based equations for prediction of P_{S9W} and P_{BW} produce f_U values within a factor of 1.5 to 2-fold, which suggests that the refinement of other model parameters may help to explain model discrepancies. The P_{BW} is also required to calculate an apparent volume of distribution (V_D), which adjusts for differences in sorptive capacity between the fish and blood. Although P_{BW} is used to estimate both f_U and V_D , the sensitivity of IVIVE model outputs to changes in V_D has not been investigated. In this study, we evaluated commonly used equations (empirically derived and composition-based) for prediction of P_{BW} and how differences in P_{BW} influence estimates of V_D . The V_D estimated using a composition-based approach remained relatively constant at $\log K_{OW} > 4$ whereas an empirical approach yielded estimates that progressively increase with $\log K_{OW}$. V_D values generated using these two approaches deviated by approximately 2- to 50-fold at $\log K_{OW}$ between 5 and 10. These differences in V_D directly translated to differences in extrapolated whole-body biotransformation rate constants and modelled BCFs, suggesting that errors in specification of V_D , rather than f_U , may contribute to IVIVE model deviations. This work highlights the uncertainty in V_D and the need for studies to investigate this IVIVE model parameter. Potential methods for experimental determination of V_D for hydrophobic chemicals are discussed.

4.17.P-Tu280 A Generic Toxicokinetic Model for Mammals: Case Study Applications for Rodents

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Chemical regulations seek to protect the environment and human health. There is a recognized need to implement New Approach

Methods (NAMs) in chemical assessments and to reduce animal (vertebrate) testing. Understanding toxicokinetics (TK) in biological systems is critical for interpreting experimental testing data and for developing models to address data gaps. In this study we describe a one-compartment physiologically-based toxicokinetic (PBTK) model that can be readily parameterized to various mammalian species requiring only a few key biological parameters, e.g., body size, lipid content. There is also a minimal number of chemical input parameters required to simulate the TK of neutral and ionizable organic chemicals (IOCs), e.g., partition ratios, biotransformation rates and pKa for IOCs. The biotransformation rates can be obtained from *in vitro* bioassays or *in silico* methods (e.g., QSARs). The model includes a novel *In Vitro* – *In Vivo* Extrapolation (IVIVE) model for incorporating *in vitro* biotransformation rate data. The model performance is evaluated in a case study with independent *in vivo* data representing a diverse range of chemical partitioning and reaction properties.

4.17.P-Tu281 Investigating Machine Learning Approaches to Predict Toxicokinetics and Toxicodynamics in Aquatic Organisms

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Chemical contaminants are continually released into the aquatic environment which subsequently leads to the exposure of organisms. This exposure of these organisms can lead to toxicity through uptake processes that are related to the kinetics of the chemical compounds. Depending on different physiological and chemical factors, e.g. species, life stage, chemical and exposure concentration, organisms can be adversely affected through TKTD processes at different levels of severity, ranging from more subtle effects like behavioural changes to mortality. In the case of pharmaceuticals, this is specifically concerning as they are designed to affect specific molecular pathways and, thus, have a high probability of unintentionally affecting wildlife. To understand potential effects, uptake, biotransformation, and elimination of chemical compounds are critical to investigate. Currently, analytical testing methods to establish the safety profiles of compounds involves the use of live animals resulting in a high cost and animal usage. Furthermore, frameworks, like the 3Rs (Replacement, Reduction and Refinement), aim to reduce animal testing in research. Thus, alternative and supporting methodologies are needed. *In silico* methods based on artificial intelligence offer one possible approach to assess chemical risk. Hence, in this work we used liquid chromatography-tandem mass spectrometry to generate toxicokinetic data from aquatic organisms (e.g. fish and invertebrates) exposed to multiple classes of chemicals including pharmaceuticals and pesticides. The data was modelled via the development and validation of machine learning algorithms such as tree-based learners and artificial neural networks to predict uptake and elimination kinetics. Models developed were also applied to previously published effect-based data to enable prediction of toxicodynamic effects. Algorithms were compared for their predictive ability to generalise to new datasets. Validated models were further investigated to understand relevant parameters that influence TKTD processes. The approach offers a novel solution to assess chemical risk and increase our understanding of TKTD mechanisms for chemicals that are present in the aquatic environment.

4.17.P-Tu282 Mechanistic Insights on Structurally Similar cVMS Using Physicochemical Properties, Bioavailability, Metabolism and Toxicity Profiles

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Regulatory and authoritative evaluations have grouped octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6), which are cyclic, volatile methyl siloxane (cVMS) substances with four, five and six dimethyl siloxane groups respectively, into one category (cyclosiloxanes) due to structural similarity with comparable physicochemical properties. For example, in 2018 the European Commission published a restriction on the use of D4 and D5 in wash-off personal care products (PCPs) in the European Union. The addition of D6 and extension to leave-on PCPs and “other consumer/professional products” for all three substances is also currently under evaluation by the European Commission. The Annex XV dossier justifies this grouping based on these substances’ “similar” chemical structures and hazard profiles (all are identified as vPvB substances, whilst D4 is also identified as a PBT substance). However, closer examination of these three substances demonstrates key differences in their behaviour from an environmental fate perspective and toxicity profiles that should be considered in regulatory assessments. A substance-by-substance approach demonstrates that although these three cVMS substances appear to have similar structures and physicochemical properties, key differences in physicochemical properties lead to differences in bioavailability, metabolism, toxicokinetic profiles and ultimately different toxicity profiles that question this grouping. This presentation provides a review of the chemical structures, physicochemical properties, bioavailability, metabolism, toxicokinetic and toxicity profiles that suggest that understanding potential for exposure and the modes/mechanisms of biological interactions are equally important for assessing the ability for read-across for this class of compounds.

4.17.P-Tu283 Investigation of the pH -Effect on the Toxicity, Uptake & Biotransformation of Metoprolol in Zebrafish (Danio rerio) Embryos, Utilizing LC - ESI - QTOFMS

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The release of emerging contaminants (such as pharmaceuticals) into the aquatic ecosystems has become an issue of concern among scientists. Most of them are frequently detected in the aquatic environment and may bioaccumulate in aquatic organisms causing adverse effects. Metoprolol (MET) is a cardioselective β -blocker, and it has been frequently reported in aquatic

environments between ng L^{-1} and ?g L^{-1} . Furthermore, MET is an ionizable organic compound (IOC). IOCs are substances whose chemical speciation (ionic or neutral form) is defined by the surrounding pH value. The uptake of the IOCs, and as a result their toxicity, is strongly affected by their speciation. However, the influence of the different pH values on the toxicity of IOCs has not been extensively evaluated in risk assessment studies. Zebrafish (*Danio rerio*) (ZFE) has emerged as a powerful alternative model organism, which is widely used in ecotoxicological research studies for evaluating the potential effects of xenobiotics on aquatic organisms. The aim of this study was to assess the influence of the different pH values of the test medium on the uptake, potential bioaccumulation, biotransformation, as well as the toxicity of the β -blocker MET in ZFE. Another goal was to determine the potential biotransformation products (bio-TPs) of MET and to assess whether the bio-TPs could contribute to the toxicity of the parent compound. For this purpose, the fish embryo toxicity test (FET) with ZFE was conducted at different pH values. The LC₅₀ values of MET at 3 different pH values were determined (6, 8, and 9) and were used for the exposure experiments of MET. For the extraction of MET in ZFE samples, organic solvents were added, and a bead-beating homogenization process was followed. Exposure water samples and ZFE extracts were analyzed by RPLC and HILIC in both positive and negative electrospray ionization mode, using LC-ESI-QTOF-MS. A target screening approach was followed for the identification of the parent compound MET whereas the detection and identification of tentative bio-TPs were performed through in-house developed suspect and non-target screening workflows. The internal concentrations (C_{int}) of MET in ZFE extracts were determined. The bioaccumulation of MET was evaluated and the bioconcentration factors (BCF) of MET were calculated. Also, the biotransformation of MET in ZFE was studied and many bio-TPs were detected. Finally, a potential biotransformation pathway was proposed.

4.17.P-Tu284 Physiologically Based Pharmacokinetic Model (PBPK) for Three Flame Retardants -TDCIPP, TCIPP, Tcep- in Animals and Extrapolation to Humans for Risk Assessment

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Flame retardants (FRs) are widely distributed in environmental samples (i.e., air, water and soil), animals and food. Dietary intake, as well as inhalation and dermal absorption are the major routes of exposure for humans. There are increasing concerns about health effects of these FRs by affecting the endocrine, immune, reproductive, and nervous systems. Among FRs, Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), Tris (1-chloro-2-propyl) phosphate (TCIPP) and tris (2-chloroethyl) phosphate (TCEP) are 3 widely used chlorinated organophosphate, which are frequently detected in humans. Because of their physicochemical properties, they are considered to have potential neurotoxicity in humans. In order to interpret the biomonitoring data in the context of chemical risk assessment, it is necessary to have a mechanistic framework that explains the distribution of FRs in the human body. Physiologically based pharmacokinetic models (PBPK) are widely used for toxicokinetic and dosimetry assessment. In the present study, PBPK models for three FRs (TDCIPP, TCIPP and TCEP) were developed in animals (adult male rat/mice) and further extrapolated to humans through allometric scaling. A 6-compartment PBPK was developed incorporating detail metabolic kinetic. Further optimization of several parameters was done for the toxicokinetic using Bayesian framework (Markov Chain Monte Carlo). Developed models were validated using experimental data with acute oral exposure and measured concentration in both feces and urine as major elimination routes. Finally, validated animal models were extrapolated to humans and were simulated for different exposure scenarios. The 3 FRs showed a similar metabolic profile (faster metabolism), and a short half-life. Most metabolites were present in plasma and brain of the mice for longer times, as predicted by the model. Even with single oral dosing, chemical stayed in the brain for a longer time, which may be one of the reasons behind observed neurotoxicity in human beings. In urine and feces, only the major metabolites BDCIPP, BCIPP and BCEP (only in urine) were detected. In further studies, we will use this model to predict the intake and exposure of these 3 FRs in humans using biomonitoring data based on urine data with reverse dosimetry technique. The current study may provide a translations tool for estimating the human health impact of FRs.

4.17.P-Tu285 Assimilation Efficiency of Cadmium (Cd) and Influence of the Exposure Pathways on Its Distribution, Toxicokinetic and Fate in the Organs of Gammarus fossarum

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Most of the studies conducted on metal bioaccumulation in freshwater crustaceans are focused on waterborne contamination and did not take into account the metal uptake through consumption of contaminated food sources. Thus, the quantification of both uptake pathways allows having a global picture of metal exposure for these aquatic organisms. To the best of our knowledge, no studies focused on the dietary bioaccumulation of Cd by assessing its toxicokinetic and organs distribution in the bioindicator species *Gammarus fossarum*. In a first step, the Cd assimilation efficiencies were assessed for two types of food usually consumed by gammarids. Thus, chironomids larvae and alder leaves were preliminarily radiolabelled with ¹⁰⁹Cd before a pulse chase feeding (2h) of gammarids. Once fed, the radioactivity content in individualized gammarid was followed along a 14-day depuration phase allowing to determine the Cd assimilation efficiencies and biological half-life of assimilated fraction. The first results show a Cd assimilation efficiencies of 60% and 52% for leaves and for larvae, respectively. In second step, gammarids were exposed for 6 days to alder leaves prior radiolabelled by ¹⁰⁹Cd, before being placed in depuration conditions with uncontaminated food for 11 days. The absence of dissolved ¹⁰⁹Cd released by radiolabelled food was monitored twice a day during exposure phase and daily during depuration phase. At several sampling time, the Cd contents were quantified in the gammarid organs, i.e. caeca, cephalon, intestine, gills and remaining tissues by gamma-spectrometry. The results show: i) a strong organotropism with difference of accumulation efficiencies among organs with caeca > intestines > cephalons > remaining tissues > gills at the end of phase of

exposure. In addition, ii) no Cd were measured in the gills confirming no recycling of ^{109}Cd in the dissolved phase and implying the absence of Cd exchanges between the contaminated organs and the gills in accordance with previous study carried on dissolved contamination. These results attest that the elimination of accumulated Cd by caeca, cephalons and remaining tissues did not request exchange processes that occur at the gills levels and imply a key role of the caeca and intestine in the fate of dietary Cd in gammarid.

4.17.P-Tu286 Toxicokinetic Modeling to Assess the Impact of Cadmium (Cd) Concentration Exposure on Metal Organotropism, Bioaccumulation and Fate in Gammarus fossarum

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The organotropism, toxicokinetic and fate of Cd in *G. fossarum* were previously assessed by following metal uptake and loss in caeca, cephalons, intestines and remaining tissues of gammarids exposed to $11 \mu\text{g.L}^{-1}$. Despite this study identified key organs for Cd bioaccumulation in acute conditions, such as caeca and intestine, the potential role of gills usually involved in metal uptake and elimination in crustaceans, was not considered. In this context, gammarids were exposed for 7 days to 3 environmentally relevant concentrations of Cd (i.e. 4, 52 and 350 ng.L^{-1}), by adding stable CdCl_2 to a fixed concentration of the radiotracer ^{109}Cd (i.e. 20 kBq.L^{-1}), allowing the quantification of very low Cd amount in small samples. Then, organisms were placed in depuration (clean water) for 21 days. At several sampling times, gammarids were dissected to recover the target organs: caeca, cephalons, intestines, gills and remaining tissues. The amounts of Cd in organs were measured by gamma-spectrometry, to obtain a Cd concentration per organ. First, the Cd distribution among the organs at the end of the uptake phase revealed that gills and caeca were the most concentrated regardless of exposure concentration. Second, the effect of water concentration on the Cd accumulation in each organ was tested by simultaneously fitting nested one-compartment TK models by Bayesian inference, applied synchronously to the 3 concentrations data sets available for each organ. The results showed that uptake rates differed as a function of exposure concentration for all organs except the intestines, in contrast to the organ-specific values for depuration rates. Finally, a multi-compartments TK model, associated to an iterative inference process, was fitted, for each concentration, to all organ datasets simultaneously to describe the fate of Cd among organs over time and find the “best” scheme of Cd management based on exposure concentration. The results showed the same pattern of inter-organs exchanges for the 3 concentrations, refining the hypotheses concerning the *in vivo* management of Cd. In addition to the absence of impact of the exposure concentration of dissolved Cd on its organotropism, toxicokinetics and fate in gammarids, this study allowed us to: i) work at environmentally relevant concentration; ii) observe that gills are the most concentrated organs in Cd; and iii) assume intestines are a pathway of uptake and loss, whereas gills are only a pathway of uptake and storage.

4.17.P-Tu287 Review of PBK Models for Fish Species of Non-Ionogenic Compounds for Use in Environmental Risk Assessment

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The global regulatory landscape is currently experiencing a shift towards the introduction of New Approach Methodologies (NAMs) encompassing *in chemico*, *in silico* and *in vitro* approaches, driven by the need to eliminate or greatly reduce animal testing to support chemical safety assessment. One of the challenges to the use of NAMs in Environmental Risk Assessment (ERA) is to derive Points of Departure (PODs) and establish these as an alternative to the traditional Predicted No Effect Concentrations (PNECs). In order to implement the use of PODs in ERA, it is essential to quantify the chemical exposure under investigation as a concentration at the target site and reverse it to the external concentration required to achieve that POD internally. In this respect, the availability of robust *In vitro* *In vivo* extrapolations and physiological based kinetic (qIVIVE/PBK) models becomes fundamental. One key approach to achieve this is the use of PBK models to estimate internal concentrations of chemicals in aquatic species based on external concentrations and vice versa, from internal concentrations in *in vitro* systems to external (*in vivo* relevant), process also known as quantitative qIVIVE. Here we present a novel evaluation of available fish PBK models to estimate the internal concentration of non-ionogenic chemicals following exposure and discuss its relevance and future applications and developments. The three one-compartment models are the most representative and well known in the field which are currently available. They differ in some input parameters such as the lipid or the water content and they consider the influence of exposure time and fish trait parameters such as the fish weight and lipid fraction amongst others. Comparisons between experimental and predicted internal concentrations values were analyzed to evaluate the performance of the models. Overall, we demonstrate that the three PBK models achieve satisfactory results in the prediction of internal concentrations for organic neutral chemicals and the error remains within a 10-fold for all models. Lastly, we consider whether the performance of these models could be improved through further developments e.g., by adding multi-compartment and metabolism considerations among others.

4.18 When “multiple” matter to deal with mixtures and other stressor effects across levels of biological organisation

4.18.T-01 Assessment of the Joined Effects of Carbaryl, Dietary Restriction (DR) and Dietary Deprivation (Dd) on the Oligochaete Species Eisenia fetida

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Toxicity studies are extensively used to evaluate the effects of toxicants upon organismal health. However, studies assessing the combined effects of chemicals and dietary restrictions are rare. For dietary studies, the focus has been on biological and ecological

parameters and seldom on the added influence of chemical toxicity. Dietary restriction (DR) and dietary deprivation (DD) have been shown to be significantly beneficial, in terms of lifespan gains and stress alleviation in nematodes. However, such beneficial effects still need to be evaluated in worm's species such as oligochaetes, especially in combination with other stressors. Our study evaluated the toxicity of carbaryl at various concentrations under both DD and To do this, we used the following dietary conditions: 100% (normal diet – 5 g oatmeal per 10 worms), 50% (or DR – 2.5 g oatmeal per 10 worms) and 0% (or DD – 0 g oatmeal per 10 worms). The following concentrations of carbaryl were used 0, 7, 14 and 28 mg/kg and effects on survival, reproduction, and biomass were assessed after 14 days of exposure. Preliminary results using the normal diet showed that carbaryl is toxic to earthworms, causing a total inhibition of reproduction in all the treatments (7-28 mg/kg). Survival data showed that the 14-day LC₅₀ was higher than 28 mg/kg, but a 14-day LC₂₀ = 11.238 (4.599-27.463) mg/kg was computed. Carbaryl also caused significant weight loss ($p < 0,05$) in the 7 mg/kg treatment. The results of ongoing experiments using the DR and DD diets will be processed and compared to those of the normal diet in due course. The findings will be discussed in light of the latest and most relevant literature.

4.18.T-02 Temporally Coupled Stress Provokes Antagonistic Response at Low Concentrations

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Recent investigations revealed that consideration of pesticide pulses, which are subject to large temporal fluctuations, is elemental in environmental risk assessment. However, effects of sequential exposure in mixtures of environmental and chemical stress are rarely investigated, although such scenarios probably constitute the most dominant scenario in nature. Additionally, gaps of knowledge regarding the outcome of interactions between stressors exist. Research suggest that time and order of exposure can modify the response of pesticides in mixture with other pesticides or environmental stress. Their role concerning the interaction of stressors, however, is so far unclear. Therefore, we conducted a full factorial, four dimensional, multiple stress exposure experiment to probe the effect of exposure time and exposure order on interactions between stressors. We tested broad combination ranges of an environmental stressor (UV-B radiation, 6 exposure times) and a chemical stressor (Esfenvalerate, 10 concentrations) on the acute effect on *Daphnia M.* under 4 different exposure scenarios. We observe that the temporal distance between two stressors has a significant effect on their interaction. Applications of low stress doses, independent of the stress type, significantly increase the EC₅₀ response of the other stressor by a scaled difference of 39% of the control ($p = 0.0069$), if the stressors were applied one day apart. This effect disappears when the stressors are administered within a longer time interval of 3 days (-16% of control, $p = 0.13$). This finding is also reflected by average MDR values (1 day time lag: MDR = 0.54, 3 day time lag: MDR = 0.93), corresponding to antagonistic and additive behaviour respectively. We conclude that low – hormetic – doses of one stressor may provoke a decrease in response of the second stressor, if both are coupled in time, thus leading to observations of antagonism at low doses. With these findings we hope to add to the discourse of why mixtures often times behave antagonistically. Moreover, the results underline how the consideration of hormesis theory and related non-monotonic dose response relationships can help understand and predict effects of multiple stress in the field.

4.18.T-03 Accounting for Individual Environmental Conditions in Assessing Pollution Exposure of a Long-Lived Top Predator Population

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Environmental stressors originating from both natural (e.g. food availability) and anthropogenic (e.g. synthetic pollutants) sources may have strong influence over extended periods on long-lived species. Consequently, environmental management needs to account for the cumulative long-term effects of multiple stressors on such species. Additionally, when population sizes are reduced with individuals exposed to multiple stressors, the behavior of individuals becomes relatively more important in determining population-level outcomes, these later being more relevant in terms of conservation actions for such species. We developed an individual based toxicokinetic (IBM-TK) model for American alligators as a case study of a long-lived top predator exposed to organochlorine pesticides. The model simulates internal chemical accumulation over the lifetime of individuals, accounting for dietary uptake, growth dilution and maternal transfer processes. The model furthermore considers the between-individual variability in pollution exposure, growth and reproduction directly stemming from food availability in these highly territorial species. Environmental conditions in lakes occupied by alligator populations (such as air and water temperature as well as food web composition) are also considered. Meanwhile, lifecycle development of alligators was modelled using a dynamic energy budget, calibrated against sparse data on growth during early life stages, nest productivity, and age at reproduction. At last, prior knowledge regarding parameter values were phylogenetically derived from related crocodile species in order to feed the Bayesian inference process leading to the parameter estimates of the whole model. Long-lived top predator populations play an important role in the structure and functioning of ecosystems. With this research we aim to contribute towards quantitative mechanistic understanding of the interactions of such species with environmental pollutants present in their natural environments.

4.18.T-04 Single Cell Analysis of Microalgae Cells After Exposure to Mixtures of Transition Metals and Organics

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For a long time, the main focus has been placed on the exposure of organisms and risk assessment of individual classes of chemicals. Since the environmental pollutant landscape is more complex than one class of chemicals, the attention is starting to

shift towards the broader chemical spectrum of mixtures, including transition metals and organics and the resulting mixture effects. Complexation to a metal can alter the chemical behavior of the organic component as well as the metal. The aim of this study was to evaluate the response of the green alga *Raphidocelis subcapitata* upon exposure to mixtures of transition metals and organics. Different endpoints, including PSII inhibition and intracellular transition metal concentration changes, were measured. This paper proposes a precise and robust approach: Single cell analysis using inductively coupled plasma mass spectrometry (SC-ICP-TOF-MS) was combined with more conventional effect analysis, including measurements of growth rate and photosynthesis (PSII) inhibition, enabling a more in-depth study of the toxicology of mixtures to algae. *R. subcapitata* were exposed to varying copper or zinc concentrations and intracellular contents of Cu, Zn, Mn, P, and Mg were measured over a time period of 24 hours. Intracellular copper concentrations increased almost 60-fold within the first 30 minutes. Upon copper exposure, no significant changes in intracellular zinc concentrations were observed. Zn exposure resulted in a slower Zn uptake with a maximum at 24 h. Furthermore, intracellular copper concentrations increased upon zinc exposure. Mixtures of pyriithione and zinc have no effect on the PSII after 2h, compared to pyriithione by itself. Copper addition showed synergistic effects already after 2 hours. Toxic effects of mixtures can follow different theoretical models, concentration addition and independent action, identifying synergism, when the toxicity of two or more components is higher than predicted with the concept of concentration addition, or antagonism, when the mixture effect is lower than the mixture prediction for independent action. CuSO₄, ZnSO₄ and pyriithione (PT) were added to algae, the sum of toxic units (TU, $TU = TU_{\text{metal}} + TU_{\text{PT}} = C_{\text{metal}}/IC_{50,\text{metal}} + C_{\text{PT}}/IC_{50,\text{PT}}$, C = concentration, IC_{50} = inhibitory concentration triggering 50% of maximum effect) of metal and PT were < 1 indicating a strong synergistic effect of the mixture on the PSII inhibition.

4.18 When "multiple" matter to deal with mixtures and other stressor effects across levels of biological organisation (Virtual Only)

4.18.V-01 Cytotoxic Effects of PAHs Mixtures in Immortalized Hepatocyte Cells of Zebrafish (*Danio rerio*)

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Polycyclic aromatic hydrocarbons (PAHs) are persistent contaminants ubiquitously present in the environment. The main source is combustion of fossil fuels from anthropogenic activities. Given their high molecular weight, low solubility and high lipophilicity, PAHs are hardly degraded, persisting and accumulating in the environment. PAHs always appear as complex mixtures of compounds in the environment but, nonetheless, research has focused mainly on the effects of single compounds, not counting with interactions effects between compounds that may result in an unpredictable outcome comparing to exposures to single PAHs. The aim of the present work is to assess the interaction cytotoxicity effects of different of PAHs, considering different proportions of each PAH in a mixture. For this purpose, *Danio rerio* hepatocytes cell lines (ATCC® CRL2643™) were used as in vitro model. ZFL cells are a robust model system for toxicity studies due to the easiness to culture and manipulate and due to their capacity to retain several of the organs' metabolic functions. To assess the cytotoxicity of these compounds, ZFL cells were exposed to different PAHs usually present in the environment, at concentration within nM to μM range to produce valid concentration-response curves. The exposures were performed using individual PAHs (Phenanthrene, Benzo(A)pyrene, Fluoranthene and Benzo(B)fluoranthene) and their complex mixtures considering different proportions of each PAH, namely: i) at mixture ratios proportional to the EC50 of each component; ii) mixing all substances at ratios proportional to the individual EC10/20; and iii) at environmentally-relevant proportions based on data in scientific literature from sediments and estuarine water samples. Cytotoxicity was carried out through Neutral red, MTT and Trypan blue assays. The Combination Index (CI) was applied to data to reveal the nature of the PAH interactions (synergistic, antagonistic and additive). Acknowledgement - This work was supported by the Project PAHMIX—Mixtures of Environmental Carcinogens: a molecular approach to improve environmental risk assessment strategies (PTDC/CTA-AMB/29173/2017), by the Marine and Environmental Sciences Centre—MARE (UIDB04292/2020; UIDP/04292/2020), by the Applied Molecular Biosciences Unit—UCIBIO (UIDB04378/2020; UIDP/04378/2020) and by iMed. ULisboa's Strategic Project (UIDB/04138/2020; UIDP/04138/2020) all financed by national funds from Fundação para a Ciência e Tecnologia.

4.18.V-02 Identification of Frequently Used Mixture Combinations of Endocrine Disrupting Chemicals in Household Chemical Products Using Association Rule Mining

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Consumers use a variety of products and are exposed to many chemicals contained in them. However, previous studies evaluating the risks associated with exposure to chemicals in consumer products have focused on individual chemicals. In the present study, mixture combination of endocrine disrupting chemicals (EDCs) in household chemical products (HCPs) was identified using association rule mining and toxicological big data. After collecting information on the product names and ingredients of HCPs from the website "Living Environment Safety Information System", we unified chemical names labeled with different names and matched with chemical identifiers. Chemicals potentially exhibited (anti)estrogenicity, (anti)androgenicity, thyroid hormone disruption, and steroidogenesis inhibition were identified using ToxCast database. The frequently used mixture combinations were identified by utilizing the association rules of the R program. Among the 11,064 products, a total of 7,603 products were finally analyzed excluding products for which no ingredient was provided or duplicate products. Among the 1,241 chemical substances, 293 substances were identified to have endocrine disruption, and these EDCs were prevalent in cleaners, synthetic detergents, fabric softeners, air fresheners, and deodorants. The most prevalent combination was ethanol, hexyl cinnamaldehyde, geraniol,

citronellol, and benzisothiazolinones. The results of this study will be useful for the management of EDCs in HCPs and future studies on mixture toxicity. Acknowledgement: This study was supported by Korea Environment Industry & Technology Institute (KEITI) through the Technology Development Project for Safety Management of Household Chemical Products, funded by Korea Ministry of Environment (MOE) (Grant no. 288 2020002960006; 1485017189).

4.18.V-03 The Hierarchy of Multiple Stressors' Effects on Benthic Invertebrates: A CASE Study From Two German Rivers

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The ecological status of surface water bodies is influenced by a variety of anthropogenic stressors, such as elevated nutrient concentrations or hydrological and morphological alterations. Furthermore, a multitude of different micropollutants, e.g. pesticides and pharmaceuticals, is released into the environment every day where they are present in complex mixtures. Both the combined effects of multiple stressors as well as the effects of mixtures of micropollutants have been addressed in previous research studies. However, often micropollutants have been analyzed separately from other stressors or have not been directly included in multiple-stressor studies. This study analyses the effects of 19 different stressors belonging to physico-chemical, hydrological and morphological stressors as well as variables representing the mixture toxicity for 42 selected micropollutants. The stressors are put into a hierarchical context according to their relative impact on the invertebrate communities. The stressor hierarchy is derived from a multiple-stressor dataset and benthic invertebrate taxalists from two catchments, the River Erft and the River Niers, in Germany. Redundancy analysis and subsequent variance partitioning were applied to quantify individual and combined stressors' effects. Significant stressors were elevated concentrations of sulphate and chloride and structural alterations as well as hydrological alterations characterized by the frequency of high flow conditions and the flow variation. Variance partitioning indicated a predominant effect of physico-chemical stress, with subordinate effects of morphological and hydrological alterations, whereas only a minor share of the explained variance could be attributed to mixture toxicity in this case study. This study highlights the importance of considering all relevant stressors in the planning of management programs to improve the ecological quality of rivers. However, this study also revealed a major challenge for multiple-stressor studies, i.e. the compilation of multiple-stressor-datasets. Hydrological data, for example, were only available for a small fraction of biological and chemical sampling sites, leading to the limited number of sampling sites analyzed in this study. Furthermore, sampling of chemical pollutants may be limited by the sampling methodology; grab sampling, as applied in this study, may be less suitable to capture the full range of relevant pollutants.

4.18 When "multiple" matter to deal with mixtures and other stressor effects across levels of biological organisation (Poster)

4.18.P-Th146 Mechanisms of Combined Stress Effects

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In polluted ecosystems, a large number of toxicants and environmental stressors may occur simultaneously generating additive, antagonistic and synergistic effects. Some stressor combinations show combined synergistic effects that go far beyond what is expected when using current effect models. This high degree of uncertainty makes it difficult to predict the biological effects of combined stressors. For many decades two concepts have been applied to predict the combined effects of toxicants: (i) Combined effects of similar acting toxicants are usually assessed with the concentration addition approach (CA). (ii) Combined effects of dissimilar acting toxicants are generally assessed using the effect addition approach (EA). However, predicting the combined effect of toxicant mixtures with different modes of action provides contradictory results. Instead, effects of dissimilar acting stressors have successfully predicted with the "Stress Addition Model" (SAM). However, additionally factors as intraspecific- and interspecific competition determined the magnitude of effects. Therefore, the aim of this presentation is to identify those mechanisms that determine combined stress effects. The various existing approaches to modelling combined stressor effects have been applied to a large number of controlled experiments and field studies. This combined evaluation reveals that the magnitude and nature of interaction can be predicted with knowledge on the environmental conditions, the state of adaptation and the toxicant mode of actions.

4.18.P-Th147 Mixture Toxicity and Interactive Effect in a Binary Ciprofloxacin-Copper Mixture to *Microcystis aeruginosa*: Focusing on Low Effect Levels of Copper

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Currently, a knowledge gap remains in the field of mixture toxicity, especially those consisting of chemicals belonging to different categories (e.g., metal-pharmaceutical mixture). Here, we employed cyanobacteria toxicity experiments to study the toxicity of a mixture made of two chemicals with very different modes of action (MoA), the heavy metal copper (Cu) and the antibiotic ciprofloxacin (CIP), using a modified ray-design. The type of interaction between different concentration levels of copper and ciprofloxacin were analyzed using the concentration addition (CA) model and the independent action (IA) model, with a focus on the impact of low-effect levels of Cu on CIP toxicity to *Microcystis aeruginosa*. IA was found to describe Cu-CIP mixture toxicity better than CA, but including the global interactive effect significantly improved the performance of CA. Antagonistic effects in Cu-CIP mixture were found in with CA. A further investigation at two pH levels (8.0 and 9.0) found that

low-effect levels of copper (EC10) did not significantly affect CIP toxicity. The implications of our findings for risk assessment and EU environmental quality standards (EQS) for single compounds within a mixture context will be discussed.

4.18.P-Th148 Use of an Aquatic Mixture Toxicity Excel-Tool in the Risk Assessment of Plant Protection Products

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In the EU the aquatic risk assessment of plant protection products with more than one active substance requires a calculation of the risk of the mixture toxicity (MixTox). This assessment, which is described in the Guidance on tiered risk assessment for edge-of-field surface waters by EFSA, is quite complex and time consuming. Risk assessors from different member states have developed a calculation tool for the mixture toxicity risk assessment. This MixTox tool is intended to 1) ease the calculation, 2) harmonise the assessment, 3) minimise the risk of mistakes, and 4) facilitate the delivery of appropriate and consistent risk calculations for applicants and regulatory authorities. The tool (“AGD_AquaMix_v1.15”) is an Excel document composed of different worksheets including 1) an introduction, 2) input of toxicity and exposure data, 3) risk assessment that follow the steps described in the scheme of the AGD and finally 4) the conclusion for the acute risk assessment. In addition to the user guidance information presented in each worksheet, a living FAQ document “FAQ_MixedTox_v1” has been developed to complement the tool and includes recommendations beyond those provided in the AGD. The MixTox tool with the FAQs have been distributed to all European pesticide risk assessment stakeholders including EU member states in the beginning of 2021 (<https://zenodo.org/record/4593676#.YZOMgucxmUk>). This poster includes examples to illustrate how to use this tool and the lessons learned from the development of the tool (e.g. how to make the mixture risk assessment simpler and more “user-friendly” when applied in practise and how to better include long-term toxicity).

4.18.P-Th150 What Are the Toxicokinetic and Toxicodynamic Mechanisms Underlying the Synergistic Interactions Between Chemicals in Mixture Exposures?

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In realistic environmental conditions, a large amount of toxic chemical substances occur together, generating interactive effects that can be synergistic, additive, or antagonistic. Due to the large number of combinations of chemicals that can occur together, assessment of chemical mixtures relies on the use of predictive models. These models, however, do not sufficiently consider the effects of mixtures, and cannot predict their interactive effects. Among these effects, synergistic interactions are seen as of greater importance for risk assessment practices, as these can increase toxic effects of a mixture to far beyond what is predicted. Synergistic interactions are the outcome of some sort of interaction between chemicals, whereby either one affects how the other is taken up to the target site (toxicokinetic) or what it does at the target site (toxicodynamic). Processes that can be affected by chemicals to lead to synergism are bioavailability, uptake, internal transport, metabolism, binding at target site and excretion. One example of synergism in invertebrates can be observed between azole fungicides and pyrethroid insecticides. Azoles (triazoles and imidazoles) have fungicidal activity in the membranes of fungi by targeting the C14-demethylase in sterol biosynthesis. In the body of an invertebrate, however, azole fungicides have been suggested as likely candidates to inhibit a wide range of cytochrome P450 monooxygenases (CYPs) or bind glutathione-containing compounds such as glutathione-S-transferases (GSTs). Both groups of enzymes have a role in the binding, sequestration and/or detoxification of pesticides. However, recent studies showed multiple cases where the synergistic effects of these azole compounds cannot be fully explained by these processes alone and thus, we do not know the full mechanistic attribution of synergism for azole exposure. My objective is to gain a better understanding of the synergistic interactive effects of azoles, and I aim to identify and model the toxicokinetic and toxicodynamic traits underlying the observed synergistic effects. My approach will include the assessment of synergistic mixture effects using multiple apical endpoints (i.e., survival, growth, development), it will include the application of a novel methodology for *ex vivo* testing of enzymatic activity in terrestrial invertebrates, and will utilize modelling of the full range of observed responses by using Dynamic Energy Budget theory.

4.18.P-Th151 Endocrine Disruptive Effects of Water-Soluble Agrochemicals Extracted From Maize and Pecan Soil

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Although beneficial in reducing crop losses, applied agrochemicals end up in non-target environments after irrigation and rainfall. When fractions of these chemical mixtures are accessible to living organisms for uptake across cellular membranes (bioavailable), they pose a threat to human and aquatic health. One of the toxicological effects associated with pesticide exposure is endocrine disruption—whereby the chemicals act as agonists and/or antagonists of endogenous nuclear receptors, including the mammalian aryl hydrocarbon receptor (AhR), androgen receptor (AR), and oestrogen receptor (ER). This study aimed to investigate the endocrine disrupting (ED) effects associated with the bioavailable fraction of agrochemical mixtures associated with maize soil in South Africa. Composite soil samples were collected in two maize growing regions—the Mpumalanga province and Vaalharts Valley—known for their extensive herbicide application. Water-soluble compounds were extracted from the soil using deionised water to mimic environmental conditions and obtain the bioavailable fraction. An *in vitro* H4IIE-*luc* reporter gene assay was performed to establish whether the soil extracts contain AhR ligands. This was followed by assessing (anti?)androgenic and

glucocorticoid activity using the human breast carcinoma cell line MDA-kb2. Lastly, the T47D-kbluc cell line was used to screen the water-soluble agrochemical residues for antagonistic oestrogenic activity by evaluating binding to the ER. The MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) viability assay was run in parallel to the reporter gene bioassays to ensure that (i) the concentrations under investigation were not cytotoxic towards the respective cell lines and (ii) as quality control to prevent false negative reporter gene assay results. Results from the *in vitro* bioassays indicated that compounds present in the water-soluble extracts of the soil did not activate the AhR or AR. However, at the concentrations evaluated in this study the soil-extracts from some sampling locations caused AR and ER inhibition, indicating ED effects. The identity of the chemicals that likely caused the effects should be further investigated by applying chemical screening to the soil-extracts.

4.18.P-Th152 Characterisation of Cumulative Risk of Contaminants to Organisms Exposed to Stormwater in Oslo, Norway

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Oslo, the capital of Norway, is situated by the Inner Oslofjord. The fjord is affected by municipal discharges, industry and boats/ships. Furthermore, various environmental contaminants enter the fjord by rivers and streams, and through stormwater. The conditions of the Inner Oslofjord have been monitored by several different monitoring programs, and data for concentrations of a wide range of contaminants have been reported in biota, water and sediment samples. The objective of the present study was to use data gathered through the “Urban fjord” monitoring programme (administered by the Norwegian Environment Agency and carried out by NIVA) to perform a retrospective cumulative environmental risk assessment (CRA), identify species groups of highest risk for toxicological effects and identify the main contributing stressors to the predicted environmental risk. “Urban fjord”-data from 2016 [2] was imported into the NIVA Risk Assessment database (NIVA RAdb, www.niva.no/radb). Concentrations in stormwater were used to perform a retrospective CRA for different species groups (taxa) including molluscs, algae, invertebrates, worms, crustaceans, and fish. Effect data for the pollutants were compiled by, and processed in, NIVA RAdb. NIVA RAdb was used to calculate cumulative risk quotients (CRQ) for the different species groups for acute and chronic effects. Metals were in most cases the main contributors to the CRQ in fish, crustaceans and molluscs, and molluscs was the taxon with the highest CRQ for chronic effects. The study shows that there is a predicted risk for biological effects in organisms exposed to stormwater entering the Inner Oslofjord. *Acknowledgement:* Financing-RCN268294 “Cumulative hazard and risk assessment of complex mixtures and multiple stressors (MixRisk)” and NCTP: NIVA’s Computational Toxicology Program, NCTP (www.niva.no/nctp).

4.18.P-Th153 Target Screening and Mixture Assessment of Chemical of Emerging Concern in the River Aconcagua (Chile)

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Aquatic environments have been widely characterised by mixtures of micropollutants worldwide and scientific evidence has demonstrated a myriad of adverse effects on aquatic life (e.g., disruption of hormonal pathways and inhibition of neuronal synapsis in non-target organisms). Nevertheless, there are knowledge gaps about the chemical and ecological status of freshwater in developing countries. The River Aconcagua basin (Chile) is a basin under high water competition by mining, agriculture, and urban areas. Several settlements and relevant national economical activities (mining, agriculture, and petrochemical industries) stretch along the river. Consequently, a diverse and complex pollution fingerprint occurs, driven by the various land uses. In this study, we used an on-site large volume solid-phase extraction sampler (LVSP) to collect 50 L of water at nine different sites (3 “reference” sites, 3 tributaries, and 3 sites along the river). Organic micropollutants were extracted and their concentrations were quantified using LC- and GC-High-Resolution Mass Spectrometry. The target-screening list comprised 824 chemicals from different chemicals groups (e.g., pesticides, pharmaceuticals and personal care products, industrial chemicals, and some transformation products). The mixture assessment was conducted applying both the toxic unit approach and the species-sensitivity distribution modelling in order to predict effects at different trophic and community levels, respectively. We quantified 153 chemicals and overall, 5 biocides, 35 industrial chemicals, 51 pharmaceuticals and personal care products, and 62 pesticides were found at one or more sampling sites. Site-specific chemical fingerprints were used for mixture assessment and our results predicted that exposed aquatic organisms (e.g., decomposers, primary and secondary consumers) in tributaries and lower part of the main river were at risk. The risk drivers were pesticides followed by pharmaceuticals. We predicted the next trophic sensitivity secondary consumers > primary consumers > decomposers > primary producers in the River Aconcagua Basin.

4.18.P-Th154 Multiple Stress Effects: From Sub-Organismic to Population Level

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In natural ecosystems, long-term detrimental effects of pesticides may occur at very low concentrations, below those considered safe by the governmental risk assessment. Mechanisms potentially responsible for this unexpected sensitivity include environmental stress-factors such as food deficiency. Here, we used *Daphnia magna* as a test organism and investigated how food

limitation – a common ecological stressor – affects the mixture toxicity of a pyrethroid insecticide and an azole fungicide. Further, we investigated how food stress interacts with insecticide-induced biochemical fingerprints. We revealed that under low food conditions, the strength of synergism between esfenvalerate and prochloraz increased with an increasing concentration of prochloraz independent of the null model. Under high food conditions and at concentrations of prochloraz $\geq 32 \mu\text{g/L}$, we observed a marginal synergistic effect with an MDR = 2.1 at $32 \mu\text{g/L}$ prochloraz and 2.2 at $100 \mu\text{g/L}$ prochloraz when using CA as a null model. In contrast, the combination of both pesticides and food stress caused synergistic effects shown by an MDR = 10.9 even at $1 \mu\text{g/L}$ of prochloraz that is frequently detected in the environment. To investigate the sub-organismic response to multi-stress, we measured metabolomic perturbations in *Daphnia magna* following a 24h exposure to esfenvalerate under high and low food conditions. In total, 160 metabolites covering the groups of amino acids, fatty acids, lipids and sugars were analyzed. At $0.001 \mu\text{g/L}$ esfenvalerate – a factor of >200 below the acute median lethal concentration (LC_{50}) – the endogenous metabolome was significantly affected. Further, the effect under low food conditions was considerably stronger compared to high food conditions. Individual metabolites showed up to 7-fold stronger effects under low food conditions. In general, the metabolomic changes were largely dose-specific and increased over seven days after contamination. We conclude that common environmental stressors can strongly increase the synergistic effects of toxicants. Further, the metabolomic changes might be a possible key to explain population-level changes at ultra-low pesticide concentrations in the field.

Track 5: Life Cycle Assessment and foot-printing

5.01 Advancement in Life Cycle Inventory Modelling: better data, more accurate assessments (Part I)

5.01.T-01 Increasing the Resolution of Parametrised LCA Models Using Machine Learning

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Due to an accelerating globalization and constant economic growth it is important to keep track of the effects human activities have on the environment. This also ensures that decision makers have the possibility to base their actions on quantitative results. One method that is used to quantify the environmental impacts of a product or service is life cycle assessment (LCA). Using machine learning to aid and improve this process is a topic that has received little attention so far, especially when compared to other research areas that try to tackle environmental crises. Given the impacts machine learning has had in many different applications over the last years, it is important to investigate if and how machine learning can be used as a tool to enhance LCA research. Two areas that were identified by the authors, where machine learning algorithms can add value compared to traditional approaches are: (1) prediction speed once the model is fully trained and (2) the possibility to train networks on a reduced parameter set using feature selection. The increased prediction speed allows the model to be used to predict data over a very large set of input parameters. This could be used to increase the resolution at which LCI data is available. It could also be used to aid LCA researchers in sensitivity analyses. This work presents a method of how data can be generated from a parametrised LCA model to create a machine learning data set on which models can be trained. It showcases this using an example LCA model for cars as a case study. The random forest and neural network models used, both show promising prediction accuracy with R^2 values of 0.994 and 0.996 respectively. While the case study shown in this paper uses a LCA model of cars, the approach can be used on any parametrised LCA model. This could allow to generate LCI data with much higher resolution in the parameter space. The downside of using machine learning is the blackbox nature of the resulting models and therefore a loss in transparency. This work aims to highlight areas where machine learning can be applied beside this disadvantage. Using this approach to generate LCI data with high spatial resolution is an area that looks particularly promising and should be investigated in future research.

5.01.T-02 Modelling Fish Products in the Big Climate Database

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Global food production accounts for around a quarter of greenhouse gas emissions. Access to information about a food's CO_2 footprint is crucial if we are to steer the production and consumption of food in a more sustainable direction. We developed a free, open access database, the Big Climate Database, of 500 of the most common foods in Denmark and calculated their carbon footprint. The database was developed for CONCITO, the Danish green think-tank. The initiative has a broad societal perspective and can be used by citizens, public authorities, and businesses alike. For this reason, the database was awarded the 2021's Nordic Council Environment Prize. Key principles of the database are consistency of modelling principles and emissions models for all crops, animals, and food processing industries in all countries in the world; completeness of life cycle inventories, avoiding cut-off of flows achieved by using EXIOBASE as background database, a multi-regional hybrid input-output database; modularity, ensured by the calculation modules contributing to the database, which can be revised, replaced and expanded. Here we present in detail the model of the fish products in the Big Climate Database. In total, there are 51 seafood products in the database. The fish module accounts only for the aquaculture production, based on the evidence that wild-caught fish is a constrained resource, i.e., it is currently not possible to increase the yield of capture-fisheries (wild fish), because the carrying capacity of the ecosystems is fully exploited (or beyond) in virtually all parts of the world. The amount of capture-fisheries has been stable for the last thirty years, while inland and marine water aquaculture have supplied the entire additional demand of fish in this period. Currently, about half of the world's fish production originates from aquaculture, in particular the aquaculture of some species, such as Salmon, Tilapia, Trout, Bream, Pike and some mollusks, such as mussels, prawn, and shrimp. These are indicated as "flexible species" The identification of the flexible (unconstrained) species is based on the regression slope for 2008-2018 in FishStat and it differentiates between freshwater fish, marine fish and mollusk. The 51 seafood products correspond to

common supermarket fish products, including filleted fishes, canned or in brine products. Packaging and other materials are therefore also taken into account.

5.01.T-03 Environmental Footprint of French Food Products

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Product environmental footprint is an eternal compromise: The assessment has to be specific, to best represent production and processing choices in the value chain. Unfortunately, this need for specific data quickly becomes an obstacle and makes the work too big to do on a large scale. In contrast, generic data offer a less expensive result, but these default values only allow for inter-category comparisons, so differentiating products within a category is impossible. This balance between specificity and simplicity needs to be overcome. For food, the proportions of ingredients in products are a determining factor for the environmental footprint and reveal intra-category discrepancies. We have developed the PEFAP calculator (Product Environmental Footprint According to Packaging data) which automatically estimates environmental impacts based on the information available on the packaging. Based on the partial list of ingredients (an ordered list, but with often unknown proportions) and nutritional data available on packaging, the algorithm finds the most likely footprint by the convergence of the result over Monte Carlo runs. From a barcode, the user obtains in a few seconds a specific footprint of the product (data table and summary web page of the evaluation). Footprints have already been calculated for the 150'000 reasonably reliable products from the Open Food Facts database. This includes a subset of 30,000 products with data that are considered fully reliable. These data are used in combination with the average quantity consumed in France for the major food categories. Unsurprisingly, the consumption of animal products has the largest impact and the impact decreases as the water content increases (with beverages appearing as the least impactful categories). But within these broad trends, significant intra-category variability is observed, sometimes exceeding an order of magnitude. This underlines the importance of going beyond a generic value for an environmental footprint and integrating the specificity of the products. The creation of this algorithm allows describing the effects of (1) diet changes (i.e. inter-category consumption changes), (2) product changes (i.e. intra-category consumption changes) and (3) their respective importance (inter- versus intra-category) to identify the most important drivers for a less impactful diet.

5.01.T-04 Data Collection in the LIFE Cycle Inventory (LCI) Analysis Phase - a Methodological Guidance Using LIFE Cycle Inventory (LCI) Blocks

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The data collection process, performed during the life cycle inventory (LCI) analysis phase, is the most resource-demanding and time-consuming step when conducting a life cycle assessment (LCA). Despite being an essential step, the methodological framework for the LCI analysis phase as described in ISO14040/44 is not detailed enough, and a clear and systematic stepwise approach guiding LCA practitioners through the data collection process when conducting an LCA is still missing. As a result, variations in the implementation of the LCI data gathering process occur, leading to different levels of detail when reporting LCI data and ultimately hampering transparency and reproducibility in LCA studies. A critical review of guidance documents and LCA studies was conducted to obtain a state-of-the-art on LCA data collection practices and based on the observed gaps, a systematic, comprehensive, and detailed stepwise framework is proposed, along with recommended methods and a flexible and customizable LCI data collection template to assist LCA practitioners with the data collection process in the LCI phase. The framework consists of three steps: planning of data collection (Step 1), data gathering process (Step 2), and LCI block finalization (Step 3). The applicability of the framework and the LCI data collection template were tested using an illustrative case of NMC811 lithium-ion batteries. The framework assisted the LCA practitioner in customizing the accompanying reusable LCI data collection template, helped structure the workflow within the LCI data collection step, and facilitated the communication between the LCA practitioner and LCI data providers. Both tools allowed a full-fledged data collection in the LCI stage and are applicable for all LCA case studies. Furthermore, they could help overcome transparency and reproducibility issues of LCI data. We call for applying these two tools when conducting LCA case studies to enhance the reliability of LCAs in the scientific community, policy, and society.

5.01 Advancement in Life Cycle Inventory Modelling: better data, more accurate assessments (Part II)

5.01.T-06 Lost in Translation? Insights From the GLAD Elementary Flow Mapping Project

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The *Global LCA Data Access (GLAD)* network is a platform hosted by UNEP for finding and accessing life cycle assessment (LCA) datasets from different data providers. But the various data exchange formats and nomenclature systems currently used in LCA pose a major hurdle for data interoperability. The main goal of this work was to consistently map the elementary flows (EFs) found in the nomenclature lists of four major LCA databases (ecoinvent v3.7; Environmental Footprint v3.0 by the European Commission; IDEA v2.2 and v2.3 of Japan; and the U.S. Federal LCA Commons, FEDEFLL v1.0.3). Six steps were carried out iteratively to establish bidirectional mapping between the four flowlists: (i) define EF lists and mapping file format; (ii) provision of source and target flowlists to be mapped; (iii) defining/refining the match criteria for mappings; (iv) generate EF mapping; (v) cross-review; (vi) finalization. The procedure involved the iteration from step (iii) to (v) until the mappings reached the desired

quality, and could be finalized (vi). EF lists were separated into two main components, referred to as ‘contexts’ (sub-/compartments) and ‘flowables’ (e.g., chemical substances). The items of the two components were mapped, and subsequently reviewed, separately. Steps (iv) and (v) were conducted with the help of two IT tools specifically developed within the project. The main result are 12 unidirectional mapping files between the four nomenclature systems, including matching criteria used by the mapper tool (e.g., by name, by CAS, by synonym, etc.). The results are discussed in terms of the level of coverage, i.e., the percentage of EFs of the source list covered by the flows available in the target list. The coverage range from around 20% to 99%. Higher levels of coverage are achieved by the larger flowlists, namely ILCD-EF v3.0 (coverage within 89-99%) and FEDEFL v1.0.3 (62-95%), on the target side. The results show that the mapping approach defined within GLAD is generally applicable, and as such potentially extendible to other lists, and it achieved significant mapping coverage. The activities performed represent a starting point towards better harmonization, and eventually the definition of a common reference EF list, for consistent mapping of inventory data and LCIA methods. In this regard, extensive involvement of stakeholders (e.g., software providers, method developers, and data providers) is essential to advance data interoperability in LCA.

5.01.T-07 Towards More Realistic Global Sensitivity Analysis for Life Cycle Assessment Studies

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Life cycle assessment (LCA) is a well-established methodology for environmental impact assessments of goods and services throughout their entire value chain. The complex global supply chains and their non-trivial effects on the environment, contain numerous sources of uncertainty and variability, which can lead to uncertain LCA results that are hard to interpret. Various LCA studies used Global Sensitivity Analysis (GSA) to gain better understanding of uncertainty drivers in impact assessments. However, only few were conducted for complete LCA models that reflect real-world conditions. In this work, we propose an efficient and reliable GSA protocol that is applicable to large nonlinear background systems, while taking dependencies between related technological and natural processes into account. The implemented correlated sampling uses real data measurements of electricity mixes in Europe, parameterization of life cycle inventories, carbon and land balancing, and adds uncertainties to markets while preserving their production volumes. Reliability of our GSA approach comes from validation of sensitivity results at all steps of the protocol. Results for the case study of Swiss household consumption indicate that only tens of LCA model inputs out of hundreds of thousands drive uncertainty in LCA scores. As a consequence, in order to reduce uncertainty and increase robustness of LCA results, only few processes need data improvement. Hence, the proposed GSA protocol allows to locate wide uncertainties in life cycle inventories that require better data quality or modeling, as well as identify processes that cause strong model response even when their uncertainty is narrow. The latter need particular attention, because they are capable of making substantial differences in interpretation of LCA results. All implementations developed in the scope of this work are freely available as Python packages.

5.01.T-08 Consequential Life Cycle Assessment of Wild-Caught Fish Products

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The food industry accounts for a sizeable amount of global greenhouse gas emissions exacerbated by a growing population and increasing consumption per capita. Life cycle assessment (LCA) is a popular method used to quantify environmental impacts in a life cycle perspective. This presentation showcases a consequential LCA of 3 seafood products which aims to assess the environmental impacts from the consequences of an increase in demand for the products. Supply of wild-caught fish have stagnated in recent decades due to the oceans reaching their ecological carrying capacity, whilst supply of fish from aquaculture has seen rapid growth. This indicates that wild-caught fish are constrained and aquaculture is the marginal source of fish which will increase its production to meet higher demand. Thus, a consequential LCA of fish products typically excludes the constrained supply of wild fish and focuses on the marginal increase in aquaculture production. This study presents a more complete consequential LCA of three products from the company Kangamiut Seafood: Fileted cod, peeled prawns and shell-on prawns. The functional unit used is 1 kg of each product. This model accounts for the consequences of increasing demand for Kangamiut Seafood products, namely that other fishing companies are displaced. Thus, we include this displacement of the average industry activity as part of the model. Additionally, aquaculture is included due to increasing the market demand for fish products which cannot be met by wild-caught fish. Consequently, the environmental footprint of the products is calculated using inputs of aquaculture, Kangamiut Seafood’s landing and processing operations as well as the displacement of the industry average operations. This enables a consequential LCA which includes the cause-effect relations on the market for fish products and can account for improvements within the constrained wild-fish supplier and thereby enabling the differentiation of different fishing methods with varying environmental impacts. In this view, the fishing company can quantify any improvements in their operations in relation to the total impact from their product in a consequential perspective. Improvement scenarios are included to assess the benefits of improving Kangamiut Seafood’s landing operations.

5.01.T-09 Quantifying Uncertainty Elements in LCI Modelling of Chemical Mixtures Used for Footwear Production

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The fashion industry is a fast-growing sector that requires the application of high amounts of chemical substances along the different production processes involved. Consequently, a large degree of pollution is generated, both in terms of ecosystem and human health. Lately, many fashion brands have realized the magnitude of environmental burdens created by their products and have thus committed to transition into more sustainable alternatives. This objective is often supported in the industry by Life

Cycle Assessment studies. However, in the long and complex supply chain of fashion products, accurate data is often lacking and difficult to retrieve. For this reason, the chemical substances can be improperly assessed leading to an inaccurate compilation of the Life Cycle Inventory. The purpose of this work is to analyse a specific case study for the production of footwear and quantify quantitative and qualitative sources of uncertainty related to the modelling of chemical substances. These uncertainties are identified in different elements of both foreground and background data systems and are quantified stochastically by randomly sampling a significant number of inventory values within their range of uncertainty. The set of values obtained are used to model the inventory for the production of one functional unit. An impact assessment for global warming potential is performed to evaluate the contribution of the single elements to the total output of the model. Results show that uncertainties in both data systems are substantial, and a wide range of different results can be obtained from the LCA of the same product. The outcome is expected to determine what is the level of confidence in the LCI of industrial products, such as footwear, requiring use of chemical substances.

5.01 Advancement in Life Cycle Inventory Modelling: better data, more accurate assessments (Virtual Only)

5.01.V-01 Ecodesign of Urban Project Using City Information Modelling

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Environmental impacts are to be taken into account as early as possible in the design stages of urban projects so as to be able to select the most sustainable design. Nowadays, more and more district digital models, modelled according to BIM (building information modelling) standards and guidelines, are produced during the conception and construction of new or renewed districts. Those so-called City Information Models (CIM) can store data about buildings, underground utilities, roads, city furniture and vegetation (positioning, dimensions, materials and sometimes technical properties). Data about buildings are already used for LCA at the building scale (Marrero et al. 2020) and have been exploited at the district scale to evaluate impact of materials used for construction (Delval et al. 2018). A consequential approach (ie. evaluating environmental consequences of a change in the existing system, here the building stock or the city) could be considered as the most relevant for ecodesign objectives but is too rarely performed (Frischknecht et al. 2016; Roux 2020). Complementary data from CIM could be used to enhance consequential LCA at the district scale and simulate various scenarios (local climate conditions, shadow analysis from building morphology, impact of public spaces, roads and underground utilities, etc.). This induces a greater complexity as far as data modelling and processing are concerned. Research on urban analysis from CIM data has shed light on numerous issues to be tackled in order to be able to perform analysis at the district or city scale thanks to a CIM, including georeferencing, level of detail management, definition of specific entities and property sets and need for generalized entities (Deprêtre et Jacquino 2021). Through this work, we aimed at identifying more precisely CIM data potential for consequential LCA at the district scale in order to propose new methods and processes and test them on case studies. A literature review is performed on eco-design and city information modelling aside with a review on existing city models. Main research potential to improve LCA of urban project are identified and classified according to the goal and scope of the study. A first field case study is exposed and major drags and opportunities to district LCA are discussed in this specific case where an existing model has been developed without considering an eco-design objective.

5.01.V-02 Energy Analysis of Air Conditioners Using Big Data and Artificial Intelligence

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In October 2020, the Japanese government announced its “2050 Carbon Neutral” vision, and as a mid-term goal, it set a target in its Global Warming Prevention Plan to reduce Greenhouse Gas (GHG) emissions in 2030 by 45% from the 2013 level. In particular, the household sector is need to achieve the largest reduction of all sectors, with a 66% reduction from the 2013 level over the remaining nine years. Among home appliances, the power consumption of air conditioners (ACs) is particularly sensitive to outside temperature and consumer’s lifestyles, and the amount of power consumption fluctuates greatly depending on the time of year. Therefore, to reduce GHG emissions from electricity, which account for about half of all GHG emissions from households, it is essential to make efforts to reduce electricity consumption by reviewing consumer’s lifestyles. The concept of Life Cycle Assessment (LCA) is used when aiming to reduce GHG emissions. LCA is a method to quantitatively evaluate the environmental impact of each life stage of a product or service, from procurement of raw materials, manufacturing, transportation, use, disposal and recycling. Through this assessment, hot spots can be identified and utilized to formulate strategies for reducing GHG emissions. In the LCA case study of ACs, it was shown that the usage stage of ACs accounts for about 90% of the environmental impact, and that the calculation results vary greatly depending on the local climate and operating conditions. In addition, the Japan Industrial Standard, which is used as a guide for the usage time in the conventional calculation, assumes 18 hours of usage time per day, which is not a realistic figure for calculation. Therefore, it is stated that it was difficult to collect the data necessary for the calculation of GHG in the existing survey. As described above, energy analysis and environmental impact assessment using existing industry data on air conditioners are based on scenario settings and do not reflect the actual status of air conditioner use, which has been an issue. In this research, we will use hourly data of 70,000 ACs and artificial intelligence to clarify the actual use of each region and climate. Then, future estimates based on future temperature scenarios will be conducted based on the clarified usage conditions, and changes in heating and cooling demand due to climate change, lifestyles and equipment performance toward the realization of a carbon-neutral society will be examined.

5.01.V-03 Global Spatialized Pesticide Application Data for Use in LCA

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Pesticides (active chemical ingredients in plant protection formulations) are widely used for agricultural pest, weed and disease control, with estimated three million tonnes applied in 2018. Hence, reliable life cycle inventory (LCI) data for emissions of pesticides are crucial for assessing as well as optimizing crop protection actions and minimizing environmental impacts across various agricultural practices, for identifying related hotspots, and for evaluating agrifood products and systems in life cycle assessment. Since pesticide emissions are usually unknown, they must be estimated from reported mass applied and treated area. However, such a global application dataset including ideally all relevant agricultural crops, regions, and crop protection practices is currently missing to generate LCI data for the wide range of marketed pesticides. The aim of this study is to address this gap by generating a global, comprehensive, and spatially resolved application dataset for currently marketed pesticides as a starting point for quantifying LCI emission data and to be coupled with respective toxicity and ecotoxicity characterization results for pesticides. The starting point is the AgroWin dataset, purchased via Bayer CropScience from a commercial data provider covering 673 crop types with 368 crop growth stages were included, with more than 1100 pesticides applied to these crops across 94 nations or regions for 2018. The dataset was first curated and harmonized to align the information. Then different statistical imputation methods were applied to fill in gaps in the reported dataset for crop growth stage, application method, and applied pesticide amount. Finally, resulting pesticide application data were combined with geospatial crop distribution maps to generate a set of global gridded maps of 5 arc-min resolution. Our global pesticide application dataset constitutes a viable starting point for generating emission and impact score information for use in life cycle assessment and environmental footprinting of agrifood systems and beyond. Furthermore, spatialized pesticide application maps inform how to improve current agronomic crop protection practices, in line with national and regional regulations.

5.01.V-04 Regionalizing Ecoinvent With EXIOBASE for More Precise Inventory and Regionalized Impact Assessment in LCA

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Different hybrid LCA approaches have been developed in the past, mainly for extending process-based LCA with input-output data. One main research area and data gap in LCA is the limited spatial detail of background data. State-of-the-art Life Cycle Inventory (LCI) databases like ecoinvent have a global coverage, but low regional resolution, with the main exception of energy mixes. In this study the industry-by-industry input-output-table of the Exiobase database is used to increase the regional resolution of the ecoinvent LCI database. For this purpose, new LCI unit process datasets are created for all regions in Exiobase, based on corresponding dataset in the ecoinvent database. The economic relation between the industry sectors and regions from Exiobase is then employed to re-link the newly created unit process datasets. It means that all processes in ecoinvent are allocated to respective regions in Exiobase and in case without a direct match, the processes from aggregated ecoinvent regions are copied for all Exiobase regions within these (e.g. RoW and RER). Because every process in the resulting “technosphere” matrix with more than 335’099 rows and columns has a clearly defined location, the supply chain includes origins of input processes as reflected by multiregional exchanges in Exiobase. Our study regionalizes the full ecoinvent database with Exiobase and thus combines to state-of-the-art databases and the different strengths. While ecoinvent provides more precise technological representation, Exiobase provides more regionalization. The result is an automatic way to derive markets for processes in ecoinvent and thereby allows utilizing regional process available in ecoinvent, such as electricity mixes, in the supply chain of all ecoinvent processes. It has to be noted that sector resolution is limited as in all MRIO studies, and important market mixes should still be improved by more specific data in the future. We analysed several cases in detail to showcase the advantages of the new database. For example, water consumption impacts of beef production, including regionalized impact assessment. While the LCI results of the regionalized inventory only changed by around 5-10% (since the process specific exchanges such as irrigation water have not been adjusted), the LCIA scores using AWARE fluctuated by up to 90% for the 44 countries covered in Exiobase.

5.01.V-05 Validation of a Conceptual Framework for Better Understanding of Environmental Performance of Product Cascades: A Case Study of Reuse of Shell Jackets

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Life cycle assessment (LCA) research on the environmental performance of circular economy (CE) measures enabling product cascades e.g. such as reuse and repair and has been criticized for not taking sufficient account of functional (in)equivalency of circular and new products, displacement rates, rebound effects, product use and user behavior. Because of this, our understanding of the environmental performance of CE is limited and, probably, overoptimistic. A conceptual framework has been developed to make it easier for practitioners to collect and structure data used for better modelling related to the mentioned limitations. Ultimately, the aim of the framework is to contribute to better understanding of the environmental performance of CE measures. The purpose of this paper is to validate the usefulness of that conceptual framework. The usefulness of the framework is validated through a case study on reuse of shell jackets enabled by “premium second-hand” stores. The framework prompts data collection on product-user characteristics such as functionality (both strict product-related properties and broader properties related to user values), use cycle duration, product lifetimes, frequency of use, price and displacement. It also offers a structure for mapping such data into a coherent and comprehensive comparison. Data is collected through user questionnaires, interviews with store managers, sales statistics and lab tests (on functionality deterioration over the life cycle e.g. water repellency). The collected data, inter alia, allows for testing the sensitivity of functional units, e.g. ownership-oriented, in-use-oriented and functionality-oriented.

Results are indicated to be sensitive to the functional unit definition which calls for sensitivity analysis in CE LCAs where several functional unit definitions could be sensible. This case study demonstrates the significance of aspects included in the framework which are largely overlooked in state of the art CE LCAs of product cascades. In doing so, it demonstrates the usefulness of collecting and organizing data which underlie these aspects through the use of the framework. Thereby, the framework seems to be an accessible and time-saving tool which allows for capturing a higher level of complexity in CE LCAs. Future research could develop it further by application in other sectors. As yet, it is a useful contribution towards better understanding of the environmental performance of CE.

5.01 Advancement in Life Cycle Inventory Modelling: better data, more accurate assessments (Poster)

5.01.P-Mo242 Comparison of Municipal Solid Waste Management Systems Using the Economic Input-Output LIFE Cycle Assessment Method

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Solid waste management is not only an environmental problem but also causes universal economic loss. Within the scope of this study, existing field data for packaging waste management systems in Avcilar Municipality of Istanbul, Turkey, is used to compare the environmental impacts of the current collection method with the alternative collection scenarios. The Economic Input-Output Life Cycle Assessment (EIO-LCA) method is used for the analysis. The field data obtained by the licensed packaging waste collection company of Avcilar Municipality through field observations. For this stage, a recording system was developed in the sorting facility and the expenditures of 18 different items such as waste amounts, fuel costs, and worker expenses. NAICS codes and expenditure items in the packaging waste sectors were matched. In this study, Scenario 1 (S1), Scenario 2 (S2), and Scenario 3 (S3) were defined as 0% separation rate (mix waste), current separation rate (1.6%), and 50% separation rate. Results show that CH₄ is the main contributor to the total GHG emission for S2, while CO₂ becomes an equally important emission for S3. The main reason of high CH₄ emission in S2 can be the increased amount of mixed waste with organic waste content. The increase of CO₂ in S2 compared to S1 can be related to several factors such as more vehicle use and related fuel consumption in S2. There is no significant change for N₂O and other emissions between different collection scenarios. Among the three scenarios, S3 has the lowest GHG emission value: 950 t CO₂-eq less than S2 and 454 t CO₂-eq less than S1. The results show that alternative package waste management systems and better practices have a high potential to reduce GHG emissions in Istanbul and other cities of Turkey.

5.01.P-Mo243 A Parameterized Model for the Evaluation of Marine Operations' Impacts During the Installation Stage of Offshore Wind Farms

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Offshore wind farms have known a growing success since 1991 in Europe due to higher and more stable electricity generation compared to onshore wind farms resulting from more constant winds and almost no land use apart from the onshore substation to connect the turbines to the grid. During the installation stage, offshore wind farms require marine operations to transfer and install their different components. These operations have potential environmental impacts. In the scientific literature, the environmental impacts associated with the life cycle of wind farms are typically estimated using the Life Cycle Assessment (LCA) methodology. However, these LCAs often lack a detailed modelling of the installation stage. In fact, the assembly stage modeled in most of these studies only accounts for the fuel consumption linked to the used installation vessels. Also, most studies tend to report the climate change impacts of offshore turbines only, despite the larger range of potential environmental impacts associated with this type of electricity production. Finally, most published LCAs are static and specific for one offshore wind farm installation and thus difficult to adapt for other geographical or temporal contexts. The aim of this study was to include an extensive modelling of the on-site installation and assembly considering the required vessels into a flexible parameterized LCA model. This model builds uponecoinvent inventories and an already existing parameterized LCA model of offshore wind farms. This model extension adds site and vessel-specific parameters associated to the on-site erection and assembly stage to the model, using the Python *lca_algebraic* library. Moreover, we go beyond climate change impacts and explore additional potential impacts categories associated with the electricity production from offshore turbines following the recommendations of the ILCD handbook. Results from this study will enable a better modelling of the installation stage of offshore wind farms and a more accurate estimation of the overall environmental impacts of these farms.

5.01.P-Mo244 Closing the Gaps in LCA of Lithium-Ion Batteries: Case Study of a Lab-Scale Battery Cell

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Battery storage systems have become a major pillar in the transformation of the energy and transportation sectors seen during the last decade. Lithium based batteries are the dominating technology in this process, making them constant subjects of sustainability analyses. Several Life Cycle Assessments (LCAs) for Lithium-ion Batteries (LIBs) have been performed over the last years to assess their environmental footprint, but the amount of primary data available concerning their manufacture remains low. This work presents a screening of the most recent environmental assessments for LIBs with the goal of identifying deviations of

results, remaining gaps and challenges, while also providing a detailed LCA of a lab-scale battery cell production. It is observed that many studies repeatedly refer to only a few disclosed datasets for their own analysis, while some others do not provide their data acquisition process in a clear manner. This creates a need for new transparent datasets in order to perform more reliable and robust assessments. The analysis hereby presented offers primary data of battery manufacturing, which contributes to reduce the data gaps in this field. Transparent description of data, sources, assumptions and results eases the understanding of the different dynamics within the life cycle of the batteries, which lastly determine its environmental profile. The hotspots identified in the baseline analysis are related to process inefficiencies, characteristic of lab-scale production. A sensitivity analysis provides insights into the potential effects of upscaling. When the manufacturing conditions reflect industrial-scale production levels, the footprint of the cells is drastically reduced, dropping to values comparable to those presented in the literature. Significant improvements in most categories have already been observed when only the throughput of cells in the dry-room is increased. Additionally, ensuring low material loss-rates will grant the largest improvements related to resource criticality. Lastly, if the upscaling goes in hand with a transition to clean energy sources, the combined benefits could bring the footprint down to a small fraction of the initial estimations.

5.01.P-Mo245 Combining Electrochemical Cell Models With Primary Data for Advancing the Assessment of Emerging Stationary Battery Systems

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While detailed life cycle assessment (LCA) models for electric vehicle (EV) batteries are available, this is not the case for stationary battery systems (SBS). The latter are mainly modelled based on inventory data from EV batteries, although their layout differs substantially. In addition, the cell chemistries typically used in SBS differ from those of EV batteries due to the lower relevance of energy density for stationary applications. Especially iron phosphate-based lithium-ion batteries, but also emerging cell chemistries like different sodium ion chemistries are promoted for SBS. However, little information about the corresponding materials and system components used in SBS is available, and the uncertainty in the life cycle inventory (LCI) models is correspondingly high. To overcome this situation, we present a complete LCI for five emerging sodium-based cell chemistries, derived from a detailed cell modelling starting from basic electrochemical parameters, relying on a modification of the BatPac dimensioning tool. The openly available tool also incorporates a new cell-specific recycling model and readily generates inventory data for manufacturing and recycling of the different cell chemistries, taking into account their individual material composition. These inventory data on battery cell level are then combined with primary data for a stationary home storage system, obtained from a piece-by-piece disassembly of an existing system. In combination, this significantly advances the state of the art in LCA of SBS and reduces modelling uncertainties. In fact, the differences to previous assessments are significant, both in terms of the battery system components (with electronic components contributing more to the total environmental impacts of the SBS than frequently assumed), but also regarding the battery cells, where high differences between different cell chemistries are obtained. Interestingly, some of the assessed sodium ion battery cells show a very promising performance and might therefore be a promising alternative to current lithium ion batteries for SBS.

5.01.P-Mo246 Towards Better Modelling of Inventory Uncertainty

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In most LCA practice, uncertainty analysis is locked in a cupboard under the stairs - part of the family, but not given the same love as the other children. Current practice lacks tools that allow us to better express the knowledge we have about the performance of systems we are trying to model. The best available option for expressing non-linear relationships is writing formulas as text strings, an approach that is not portable across software systems, does not allow for software best practices such as testing and continuous integration, and does not scale beyond a few datasets. In this poster, we introduce a set of innovations available as free and open source Python software, with a reference implementation in the Brightway framework. The first step is to recognize that not all inventory calculations can or should be done by the LCA software. Instead, we introduce defined interfaces, which allow for dataset-specific values to be generated by outside software or web services on-demand during the static or stochastic LCA calculation. In a case study, these interfaces allowed for two-way communication between LCA software and an Aspen simulation. We also extend the concepts of “pre-sampled data” to make such arrays the core of iterative LCA calculations. By supporting the use of arrays of data, instead of a single static vector of “best-guess” values, one can incorporate both measured population data and multiple possible scenarios in a single, intuitive structure. These arrays can express correlation across datasets or even databases, and have no inherent limitations in their flexibility. In many cases, they will have substantially higher quality than fitted probability distribution functions. Finally, we define an open data format, based on the Datapackage standard, which allows for static and stochastic inventory data and metadata, including population and scenario data, to be exchanged across computers and even LCA software systems. This datapackage also separates the computation of LCA results from inventory data entry, allowing for cloud-based calculations. Although the innovations described here have so far only been implemented in the Brightway ecosystem, we encourage other developers to borrow our code and ideas to allow the community to substantially improve the quality of our inventory models, especially under uncertainty. Rigorous uncertainty assessment is a critical component of comparative LCA.

5.01.P-Mo247 Quality and Temporal Relevance of Lithium Supply Data for Life Cycle Assessment

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Lithium-based batteries are increasingly being implemented for storing energy, both in transportation and stationary applications. As battery manufacturing matures and becomes more efficient, the environmental burdens of these batteries shift upstream, for example to the lithium supply. The majority of the current global lithium supply comes from two sources – spodumene mined in Australia and brines extracted in Chile. In this study, we review existing life cycle assessment literature on lithium production regarding data completeness and quality, as well as temporal and geographical relevance. Preliminary results indicate that the currently most used datasets in life cycle assessment studies of lithium-based batteries lack quality and representativeness of current operations. To address these gaps, this study compiles several new datasets for lithium production representing different geographies, technical processes, and lithium grades. First, we compare the inventory data of other existing lithium supply datasets, both older and newly compiled, regarding their quality and representativeness. Second, we look at future scenarios for lithium supply based on global proven reserves and analyze the influence of changing grades on future environmental impacts. Third, we examine the potential for reducing environmental impacts from the lithium-supply chain by linking all electricity inputs to renewable sources. Finally, we use the various lithium datasets compiled in this study to update the results of a giga-scale lithium-ion battery manufacturing in a recently published study. We focus on climate change and mineral resource use impacts. Additionally, to inform a growing debate in scientific literature around the water use impacts related to brine and freshwater extraction in water-stressed regions of the world, such as the salars in South America, we use regionalized water use assessment indicators to further assess the burdens of battery production from water use perspective.

5.01.P-Mo248 FAIR Data in LCA - Principles to Practice

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The key to increase the reliability of a LCA is to increase reproducibility, interoperability and accessibility of its data. Implementation of data management in addition to integration of data services can facilitate the development of FAIR LCA inventory data. We present and discuss the type and appropriateness of different data structures and technologies to manage the diversity, size, and complexity of current and future data sets that are relevant in LCA context. We show how implementing a data management plan for LCA studies supports the FAIR data principles. For example, ensuring data description (metadata format and documentation), legality (licensing, confidentiality, ethics) and storage (repositories, publications). With respect to interoperability, we introduce the benefit of linking LCA databases to the semantic web. Semantic web supports the ability of computer systems to exchange data with shared meaning. Integration of LCI data to the semantic web provides opportunities to work with data outside the domain, thus improving quantity and type of data availability. This work contributes to the definition of best practice measures to support the development of database infrastructure for LCA.

5.01.P-Mo249 Site-Specific Life Cycle Inventory Modelling of Lithium Carbonate Production From Brines

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The transition towards low-carbon technologies requires metals. One of them is lithium required for energy storage, in particular for the electrification of the mobility sector. However, lithium extraction is associated with environmental impacts. The energy-intensive production of lithium carbonate contributes to climate change, while the lithium extraction from brines affects water scarcity in arid areas and hence, fragile wetland and lake ecosystems. A specific focus in science has been lithium extraction at the Salar de Atacama in Chile where life cycle inventories (LCI) exist. However, due to the increasing demand for lithium, brine operations in Argentina, the U.S., and China have started to produce lithium carbonate. Processing is adapted to the brine chemistry since impurities, such as magnesium or boron, highly impair the lithium recovery rate during processing. Hence, the question rises if the existing LCI still reflect environmental impacts of lithium carbonate from brines. This study aims to quantify the environmental impacts of 1 kg lithium carbonate (battery grade). We modelled LCIs for five brine operations in Argentina, Chile, and China. Scientific literature, patents, and company reports were used to develop processing sequences. A detailed analysis of relevant processes yielded the quantification of resource consumption as well as waste production. The ReCiPe endpoint method (2016) applying the hierarchic perspective was used to assess environmental impacts. Since transportation heavily contributes to health impacts from climate change and fine particulate matter formation, these impacts are crucial when assessing impacts of lithium carbonate production from brines. The variability of both impact categories is substantial. The Chinese salt lake has highest impacts due to heat and electricity demand. Lithium carbonate produced from the four brine operations in Argentina and Chile have lower impacts. Their impacts are dominated by the chemicals demand. Hence, highly resolved LCIs as resulting from our research are required to (1) identify environmental hotspots, and to (2) find mitigation potentials within the lithium supply chain. These modelled LCIs are furthermore crucial to (3) assess the contribution of lithium carbonate production to the total environmental impacts related to Li-ion batteries and to (4) create a solid data base for the future evaluation of secondary lithium carbonate production.

5.01.P-Mo250 LCA on Different Production Routes of Calcium Zincate Synthesis

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Calcium zincate may be used in zinc anode of an alkaline electrochemical generator, heterogeneous catalyst for the production of biodiesel or antifungal products. The conventional reactions which have been studied in the synthesis of calcium zincate are: 1) Hydro-chemical route, 2) Hydro-thermal route and 3) Hydro-mechanical route. These processes present several drawbacks, such

as requiring long reaction time or having numerous energy intensive steps. The Hydro-thermal route seems to be the most competitive conventional reaction for calcium zincate production at laboratory. The presentation will show the life cycle environmental impacts of a new and unusual synthesis which is part of the field of mechanochemistry: Hydro-micro-mechanical route. It will be compared towards the hydro-thermal route at a lab-scale. This new process, patented by EASYL (2016), may be operated continuously and at industrial scale. In addition, its environmental impacts will be assessed for the production of Calcium Zincate crystals for 7 different scenarios. The results will be forecasted for 2030 and 2050 taking into consideration the European electricity mix modifications according to SDGs Roadmap. Finally, this comparison will be complemented with an economic analysis and an optimisation of the transportation strategy.

5.01.P-Mo251 LCA Data Collection for Decision-Making in Process Selection of Conversion of Woody Biomass Into Sugars

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Woody biomass could potentially become a viable raw material for the future sustainable chemical industry. For this, a suitable regulatory framework must exist, that would create favourable economic conditions for wood biorefineries. Such policies must be developed on the basis of scientific evidence, in this case - data supporting the environmental advantages of the bio-based feedstocks to the chemical industry. The most suitable methodology for comprehensive evaluation of environmental performance of technologies is life cycle assessment (LCA). European Commission's report on environmental Impact assessment of bio-based products proposes use of modular approach when collecting life cycle inventory data according to life cycle stages of a bio-chemical. In this review, we collected process data on existing semi-industrial processes of wood fractionation and attempted to match them with LCA datasets available in open sources in order to compare environmental performance of different methods. It has been revealed that the majority of the openly available studies do not contain transparent inventory data and, therefore, cannot be verified or re-used. Lack of inventory data also prevents comparison between studies of the same processes performed with different evaluation methods or using different system boundaries. While it is frequently argued that revealing full inventories is impossible due to issues of commercial sensitivity, since a full inventory may be reconstructed to reveal technological details that may form the basis of a competitive advantage, the lack of inventories is hampering transition to sustainable technologies, as decisions on policies to take technologies forward rely on science-based evidence. It is, however, possible even today to represent processes as input-output black-box models, such that any commercially sensitive details are not made public while the important environmental data over the whole supply chain is available. This issue is discussed within the context of full digitalisation of the chemistry value chain, which is likely to be reflected in forthcoming national and regional policies.

5.01.P-Mo252 Unravelling Modelling Strategies of Algae Systems: Towards Improved Transparency and Interoperability in Life Cycle Inventories

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Life Cycle Assessment (LCA) has been used for more than 20 years to assess the environmental impacts of algae systems. The diversity of algae species, cultivation techniques, and processing pathways has led to unique algae system designs. In addition, the methodological choices made by practitioners in the goal and scope and Life Cycle Inventory (LCI) increase the variability of LCA results. This study introduces a framework to enhance the transparency and interoperability of data inventories and therefore improve the comparability of LCA results. An extensive literature review was conducted to identify the modelling approaches used to mitigate the environmental impacts of algae systems over the past 20 years. The outcomes of the review served as basis for the development of the LCI framework. The results of the literature review showed that the main strategies used to mitigate the environmental impacts of algae systems include the consideration of multiple products, the use of the bioremediation potential of algae, and the design of closed-loop systems. Although seasonality influences the algal biomass content and productivity, temporal variations were rarely considered in data inventories. Finally, the analysis of the methodological choices highlighted the strong link between the functional unit, modelling perspective (attributional versus consequential), and method to solve multifunctionality. The modular LCI framework builds on Brightway2 and uses additional Python packages to include seasonal variations into LCI, facilitate co-products management, and simplify the linking of foreground and background systems. A dynamic algae growth model generates seasonal data for an open raceway pond cultivation system. Foreground and background scenario development allows the assessment of various algal biorefinery designs with different biogenic carbon flow dynamics. The algorithm was tested using data collected in a pilot scale *Spirulina* cultivation facility in the frame of the European SpiralG project (BBI-H2020). The algae dynamic growth model can be adapted to any micro- or macro-algae system and the flexibility of the LCI framework allows to conduct prospective LCA studies. Therefore, this tool could be used to support the development of any algae-based products from laboratory to commercial scale.

5.01.P-Mo253 Using a Regionalized Plastics LCA Database to Assess Environmental Impacts of Elastomers in Lightweight Applications

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In the context of a global commitment to mitigate climate change, the automotive industry is forced to drastically reduce the carbon footprint and energy consumption of its fleets. For this purpose, elastomers can be used, as they reduce the weight and thereby energy consumption of each vehicle. To evaluate the application of novel elastomer materials, Life Cycle Assessment (LCA) is a powerful tool to determine the environmental impacts of products and services, providing a holistic view across environmental impact categories and life cycle stages. However, to provide a reliable decision basis for LCA practitioners, LCA

studies for the chemical and plastics industry require a large amount of data on chemical products and processes across global production and utilization chains. Gathering this data is especially challenging for industries with highly complex and globalized production chains, like chemicals and plastics. To support decision making, we developed a novel LCA database compliant with the ISO standards 14040 and 14044. Our chemical and plastics database contains approx. 1000 chemicals and plastics in up to 190 regions. The database is derived from a regionalized bottom-up model of the global chemical and plastics industry and is based on technical data as well as market and trade data. Thereby, the bottom-up model enables chemicals to be tracked throughout the global economy. Our bottom-up approach starts at the chemical plant level, linking information about the production site, production volume and production technology to detailed technical models for each production technology. Subsequently, these individual plants are allocated to integrated production sites and national, technology-specific or supplier-specific production mixes. Moreover, national consumption mixes are calculated by combining national production mix information with trade data. In this presentation, we use the novel LCA database to conduct an LCA study for the application of novel elastomer materials based on renewable resources, such as CO₂ and biomass. The work is part of the public project ReLEA, funded by the German Federal Ministry for Economic Affairs and Energy.

5.01.P-Mo254 Virtualization of Production Processes: An Innovative Approach for the Life Cycle Inventory and Dynamic Life Cycle Assessment

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Life Cycle Assessment (LCA) is used as a tool to assess the environmental impacts of a product, process or activity throughout its life cycle providing support for product eco-design. The Life Cycle Inventory (LCI) is the second of the four phases of the LCA methodology regulated by the ISO 14040 and ISO 14044 series of international technical standards and represents the most energy and time-consuming phase of LCA. The complexity of the LCI is one of the factors that makes LCA studies difficult to carry out within the business because it requires specialists capable of conducting the analysis, a strong collaborative attitude of management and a factory staff able to conduct the collection of primary data in an effective manner. In this context technologies involved in Industry 4.0 give a comprehensive view on how companies are organized and let acquire precise data on production activity. The analysis of these data, together with the development of new digital paradigms, represents an exploitable new focus to make the production more efficient, profitable and sustainable. The potentiality of this comprehensive data collection drives the present research project to define a method that integrates the Life Cycle Assessment with the availability of data provided by Industry 4.0. Specifically, is focused on the Digital Twin (DT) concept defining a procedure to develop a physical-to-virtual connection system that will lead to a dynamic Life Cycle Inventory construction as well as a dynamic evaluation of environmental impacts related to the production processes. Research results illustrate a framework for the construction of the digital twin system to make the Life Cycle Assessment dynamic and comprehend the improving transparency, completeness and interconnection between product, company and Life Cycle Inventory.

5.01.P-Mo255 A Triple Bottom-Line Evaluation of the Production of Animal Feed From Food Waste: A Life Cycle Assessment Approach

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We perform a triple bottom line (TBL) assessment of the environmental, economic, and social impacts of the production of animal feed from food waste (FW). Animal feed production scenarios (SRs) are compared to the current options of sanitary landfilling and incineration with energy recovery. Within TBL assessment, the environmental bottom line (BL) applies a gate-to-grave life cycle assessment (LCA) approach considering a functional unit of 1 ton of FW. The system boundaries are expanded to account for animal feed production savings and recovered energy. The economic BL is calculated through a cost-benefit analysis of the total costs and profits associated with the given technologies. The social BL relies on SWOT (strengths, weaknesses, opportunities, and threats) analysis to identify the key strengths, weaknesses, opportunities, threats, and strategies of the SRs associated with waste management systems. The analytic hierarchy process (AHP) is adopted to integrate and prioritize the alternatives based on the pairwise comparison techniques inherent in this method. The AHP ranked the animal feed production scenario as the optimal scenario, with a 45.01% preference over the other alternatives. Environmentally, the animal feed production scenario scored the worst in the climate change human health impact category due to the heating energy consumed during feed production and the low nutrient recovery rate (13.5%). However, further sensitivity analysis of this rate justified the above conclusion. Financially, the incineration scenario was the most economical scenario, followed by the animal feed production and landfilling scenarios. Socially, the animal feed production scenario scored the best in most indicators, while the incineration scenario scored the worst. All the assessments provide a basis for policy-makers to evaluate new technological FW management advances to improve current FW management systems.

5.01.P-Mo256 Prospects of Integrating Ecosystem Services Into Life Cycle Assessment: Application to a Mediterranean vineyard

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Life cycle assessment is a powerful tool to compare the environmental impacts of different systems along the stages of agriculture production. However, when it comes to representing the multifunctional aspects of agriculture, LCA has shown some limitations

[1]. Indeed, if a conventional farming system provides, in general, higher yields than agriculture with organic/soil conservation practices, then the environmental impacts of conventional farming are then more diluted into an agricultural product than with an organic farming system. Some of this misrepresentation is due to the coarse resolution of spatial data used in LCA (from archetypes to regional and/or national scales), some of it is due to methodological shortcomings such as the integration of nature's benefit into farming systems in LCA. Recent studies have proposed several frameworks for including ecosystem services in LCA [2]–[6]. However, there is yet a consensus on a common method despite the recent research advances. Today, there is a need to understand the state of the art of the proposed methods and whether implementation difficulties stem from data availability and/or accessibility, process modelling, and/or both. In this study, we present the state of the art from previous frameworks proposing the integration of ES into LCA methodology and tested recent developments on a specific case study: a Mediterranean vineyard Roujan. In this research, we evaluated several ecosystem services (water yield, erosion and carbon sequestration) with various spatially-explicit modelling techniques (from biophysical modelling with the LandSoil model [7] to more empirical models like InVEST). The objective of using spatially-explicit modelling is to improve the representation of biophysical fluxes in the LCA modelling chains. To test the different existing methodologies, we have chosen Roujan, a Mediterranean vineyard in the south of France that has been monitored for several years. This site is also representative of the Languedoc region where vineyards are the main agricultural activity. We then compared several input datasets, ES methods, and their respective impacts in LCA assessments. At this stage, the ES that were assessed are: < u1:p> - Carbon sequestration due to land-use change and land management with the InVEST model< u1:p> - Erosion due to different land use and land management with the LandSOIL and InVEST models< u1:p> - Water yield with the InVEST model< u1:p> The results presented offer a new perspective on how and why ecosystem services should be integrated into LCA assessments, especially for achieving a carbon-neutral society by 2050 in the context of achieving several SDGs.

5.02 LCA estimating trade-offs of emerging bio-based products and their uses

5.02.T-01 Effect of Environmental Prioritization in the Design of Biobased Supply Chains

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Polyethylene terephthalate (PET) is one of the most used polymers worldwide, but its production relies on fossil-based feedstock. PET is made through the polycondensation of terephthalic acid (PTA) with ethylene glycol (EG). Both monomers are produced from the naphtha cracking; however, technological advances have made possible their production out of biobased resources. EG is produced from ethylene that can be synthesized from bioethanol using different biomass (food crops and lignocellulosic biomass). The technology for biobased EG is already commercially available, mainly using sugarcane. On the other hand, para-xylene (PX) is the main building block for PTA production, but its production still relies on naphtha cracking. Different pathways for PX production are available, from commercial scale (GEVO process) to lab-/pilot-scale (Alders-Diels reaction). All these technological pathways use biomass (corn and sugar beet) to produce PX. From the economic perspective, these biobased alternatives seem feasible, especially in the current lack of support to fossil-fuel companies and high oil prices. However, the environmental benefits of these alternatives compared to the fossil-counterpart are still unclear due to the lack of knowledge on the environmental impact of biomass feedstocks (greenhouse gas emissions – GHG and other impacts), and the complexity in the design of the supply chains (SC). This study aims to understand the link between the environmental impacts of biobased materials (using the 100% biobased PET as study case) and the design of biobased SC that includes economic and environmental criteria. The analysis focuses on the European context. Life cycle assessment (LCA) is used as method to quantify the environmental impact of the biobased PET production using sugar beet for EG and *Miscanthus* for PTA. The feedstock selection for both monomers was based on the technological maturity (e.g., production of bioethanol from sugar beet) and the future relevance (e.g., *Miscanthus* is a non-food crop with high biomass yields and benefits for soil). The life cycle optimization (LCO) methodology is used to establish the link between the economic and environmental performance of the biobased PET SC. The accounting of environmental impacts in optimization models for designing SC helped identify the impact categories (global warming, fossil depletion, eutrophication) with the highest contribution to the abatement of emissions in the whole supply chain.

5.02.T-02 Life Cycle Assessment of Brown-Seaweed-Based Plastic Production and End-Of-Life

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The production and end-of-life of fossil-based plastics threaten the environment. While some bio-based plastics are presented as a potential solution to avoid fossil-based plastics, these usually have a high impact on land-use change. Seaweed is compound of biopolymers potentially used to produce plastic. Moreover, seaweed cultivation has environmental benefits and does not need land resources to grow. This study uses alginate, a polymer in brown seaweed, to create a bio-based plastic. This analysis aims to assess the production and end-of-life of this bio-based plastic using a consequential life cycle assessment (LCA). Different scenarios will be analysed: Incineration, recycling and biodegrading the bio-based plastic. The preliminary results show the impacts of producing this bio-based plastic, without considering the CO₂ uptake of seaweed cultivation and the end of life of the bio-based plastic. These preliminary results show that electricity is one of the highest impacts. The final results will include the CO₂ uptake of seaweed cultivation in the sea and different end-of-life scenarios, maintaining the carbon balance.

5.02.T-03 Py-Lca-Bio Open-Access Python-Based Integrated Platform Toward Fully Automated Life Cycle Assessment and Sensitivity Analysis, a Biorefinery CASE Study

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Lack of transparency in life cycle assessment (LCA) analysis forecasts a wide range of LCA results, for example, the global warming potential (GWP) in the production of chemicals from the biorefinery may vary between a 50-80% reduction when

compared to conventional fossil-based chemical productions. One explanation for the difference in LCA results is the disparity in the utilized established assumptions to generate the LCA models and interpretation of the LCA results. Aside from that, the inventory computations are non-transparent, single-use, case-specific calculations that are not performed based on an open standard platform. Furthermore, manually uploading data to LCA software results in a time-consuming and error-prone LCA. Further methodological research may be necessary to improve the LCA results' transparency, reproducibility, and interoperability. The study aims to bridge this gap and develop an open-source python-based platform for extensively automating LCA study, including computing inventory and performing local and global sensitivity analysis of LCA results. This platform will thus allow for quick, free, and transparent biorefinery LCA study and local and global sensitivity analysis and uncertainty quantification of LCA results for several indicators concomitantly. The developed platform, named as PY-LCA-Bio, has four primary objectives: Setting up a framework for transparent and reproducible LCA studies of a biorefinery. Having an extensive facilitative function: Since the developed platform is considered an open-source platform, other researchers are encouraged to contribute to the project by modifying and expanding the platform's capabilities to meet their needs. Developing interoperability function: PY-LCA-Bio can swap formats and interface with other software. Increasing the contribution to sustainability analysis and improvements: PY-LCA-Bio, like all other open-source software, is entirely free to use and has no hidden costs. By performing the LCA with PY-LCA-Bio, it is possible to build life cycle inventory, perform LCA and perform the Global and Local sensitivity analysis along with the uncertainty analysis with just one click. Importantly, the produced inventory in PY-LCA-Bios is not limited to usage in PY LCA Bio. It can be used for the LCA of biorefinery on any platform.

5.02.T-04 Challenges Aligning LCA Results Across Bio-Based Industries

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This paper reviews the challenges faced in applications of life cycle assessments of biobased production, and suggests how to deal with this challenge. The vision of the European bioeconomy is 1) to strengthen, scale-up and diversify the biobased sectors, 2) to deploy local bioeconomies across the whole of Europe, and finally 3) to understand the ecological boundaries of the bioeconomy [1]. The complexity of the biomass resource supply and demand makes it difficult to estimate what is the sustainable use of bioresources, and biobased sectors are predicted to grow, and thus increasing the demand for bioresources. This increases the demands for decision support safeguarding that the bioeconomy is operating within safe ecological limits. The paper investigates implementation of LCA's in research and innovation studies funded by the European Union, as well as current initiatives towards harmonising LCA practises. Central questions are *key challenges and needs with respect to the development of an LCA practises?* that can guide a sustainable development of the European Bioeconomy, and *how to achieve a balancing of two major strategies of standardisation vs a science-based approach to LCA.* The paper identify 7 core areas with challenges and needs, and conclude that LCA of biobased production faces challenges underlining the need to develop an overall framework for assessment of biobased products. This framework need to depart from the current standardisation trend in regulatory LCA and turn towards a science-based approach: 1) using integrated assessment models for scenario modelling, 2) building on consequential modelling of competition for biomass and land, 3) developing harmonised models for dynamic and time dependent carbon accounting, 4) derive sets of biodiversity and socioeconomic indicators, and 5) establish a harmonised guide to uncertainty analysis.

5.02 LCA estimating trade-offs of emerging bio-based products and their uses (Poster)

5.02.P-Mo257 Assessment of Corn Cob Particleboards - Thermal and Environmental Performance

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Corn cob is considered an agricultural bio-waste with potential to be incorporated in the building sector, as a thermal insulation material. Nonetheless, more research is needed to establish a detailed analysis regarding building materials' thermal performance, when this waste is reused as raw material to assess their sustainability profile. This study aimed at evaluating the thermal behavior and the environmental impact of two distinct corn cob particleboards using different glue binders: Polyvinyl Acetate (PVA) and Fabricol AG222 (FAG222). An experimental study was performed to analyse the particleboards' thermal performance, allowing the estimation of the thermal transmission coefficient. A sustainability analysis was then carried out using a lifecycle assessment (LCA) tool according to ISO 14040 and ISO 14044. The functional unit "mass of material required to provide a thermal resistance (R) of 1 m²C/W" was considered for the calculation of the environmental impacts. The production and disposal phases were considered, incineration and landfill being the end-of-life options considered. Both particleboards have shown potential to be used as a sustainable building material for the thermal insulation of walls, FAG222 showing similarities with the current commercial insulation materials. Both options display environmentally friendly profiles, albeit the particleboard with PVA offers enhanced results for the landfill scenario. The estimation of land use change is the next step to be performed, in order to understand if a process scale-up would enable a viable path, consistent with circular economy principles. This research work is thus a contribution to the scientific knowledge regarding the valorization of agriculture wastes and by-products as potential eco-friendly building materials.

5.02.P-Mo258 Analysis of Key Issues and Methodological Guidance for Life Cycle Assessment in the Field of Circular Bioeconomy

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The use of biowaste, biobased residues and CO₂ as feedstock for bioeconomy is promoted as an alternative to cultivated biomass that increases the pressure on land resources. Using alternative substrates for products with a higher added value compared to

waste treatment technologies, is part of the circular bioeconomy (CBE) concept. It is often expected that high-value use of alternative feedstock is environmentally beneficial, but further research on the performance of CBE-inspired products is needed. When conducting life cycle assessment (LCA) in the field of bioeconomy, the practitioner is confronted with specific methodological and data-related challenges regarding the assessment of CO₂, waste and by-products as feedstock and the modelling of circularity types that cannot be grasped with existing models (e.g. closed, open loop recycling; product cascading). Based on a literature review this study analyses these key issues, assessing CBE case studies and general methods used in circular economy. For the literature review, search queries and selection criteria were developed for two key issues and for the application areas. The analysis of selected studies was guided by the question whether the methods are suitable for decision support and whether they are applicable to biobased circular systems. The key word search resulted in a significant amount of articles (46% case studies, 54% general methods), most of them addressing circularity evaluation. The analysis of the selected literature identifies whether a clear differentiation between feedstock types is made and which methods are applied to evaluate their impacts. First results indicate that CBE case studies use the approach of “alternative fate” when biowaste are used, but apply other approaches for by-products. The next analysis step will link approaches with the decision context stated in the study and identify methods that support decision making. Evaluation of the selected general methods for the handling of feedstocks indicates that specific methods are available for different feedstock types. Also, rules for a consistent handling of the feedstock input side and the product output side are available, recently translated into generalized equations. However, their applicability for biobased feedstock is demonstrated for few examples only and the general suitability is not sufficiently discussed. From the results conclusions will be drawn about the necessity of improvements of the current modelling practice. We will further conclude about the suitability of methods for decision support and transferability of general methods to CBE applications. With this study we contribute to a more consistent modelling of alternative feedstocks and circularity in the emerging field of CBE.

5.02.P-Mo259 Environmental Evaluation of Phosphorus Recovery Processes From Wastewater Sludge Using LIFE Cycle Assessment

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In addition to the sanitary aspect of wastewater treatment, another function of wastewater treatment plants is to recover phosphorus from wastewater in order to limit the eutrophication phenomena that can severely damage ecosystems. This extracted phosphorus is mainly concentrated in the wastewater sludge. Nowadays with the ban on land spreading of this sludge, treatment options such as incineration are becoming more and more common. One of the problems with this method of sludge valorisation is that it breaks the cycle of phosphorus, which ends up in the residual ashes, often sent to landfill. In this context, the Interreg Phos4You (P4Y) project addresses the phosphorus (P) challenge. P is an essential nutrient for all living organisms. Though it is a finite resource on earth, P is largely wasted today. The EU acknowledged this by adding Phosphate rock to its list of critical raw materials in 2014. P4Y aims to develop technologies for phosphorus recovery from sludge. Various processes are developed and promoted during this project, ranging from thermo-chemical treatments to bio-acidification followed by precipitation directly integrated into the water treatment plant. A life cycle assessment (LCA) was carried out on four of these phosphorus recovery processes to quantify the environmental impacts of the processes and to identify the most polluting steps within each process, in an eco-design approach. The particularity of this LCA is to include the wastewater treatment plant in the system boundaries and then not to opt for the zero-burden assumption for the sludge. Sewage sludge is therefore not considered as waste and has an environmental cost. In order to avoid allocation problems between the water treatment function and the sludge production function of the treatment plant, two options have been studied. The first methodological approach is the extension of the system boundaries to take into account the wastewater treatment plant in addition to the treatment of the sludge produced. The system studied with this option in the framework of the Phos4You project therefore has two functions, wastewater treatment and phosphorus fertiliser production. The second methodological approach used is the avoided burden. This approach studies a system with only the wastewater treatment function and considers the fertilisers produced by recovering phosphorus from the sludge as avoided mineral fertiliser production. The environmental study showed that two of the four recovery technologies had an environmental advantage compared to the baseline scenario of sludge incineration. Acknowledgements - The authors would like to thank the Phos4You project (INTERREG VB NWE, n° NWE292), partly financed by the European Regional Development Funds, and the Walloon Region.

5.02.P-Mo260 Towards Including Non-Gaseous Climate Agents in Impact Assessment Methods for LCA of Wood Products in Canada

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Climate research made some progress in terms of quantifying non-gaseous climate drivers such as albedo modification from land use change and/or transformation and adding their influence into climate metrics, as these impacts have been shown important, particularly in boreal forests. Current life cycle assessment (LCA) methodologies exclusively include gaseous forcing agents, using equivalencies (potentials and timeframes). A few papers proposed a methodology to incorporate albedo modification into established metrics, but they do so by emulating a gaseous behaviour i.e., using characterisation factors (CFs) converting an instantaneous biogeophysical forcing agent (albedo) into a greenhouse gas equivalent. Some have noted the disparities between current climate metrics and the prompt nature of albedo. We think that albedo must be included differently into LCA in order to better represent the dynamics of forest management (steady-state, increased or decreased harvest rates, etc.). Our goal is, by using albedo change in Quebec forest management as a case study, to demonstrate the validity of empirical inclusion of non-gaseous climate drivers in LCA. We simulated the regrowth of a typical forest stand using age-albedo data for each cover types surveyed

by Quebec's forest ministry. This way, after using a dynamic tool such as DYNCO2, developed to implement the dynamic LCA method, we can approximate the cumulative forcing as the added influence of albedo modification during the life cycle of a hectare of forest during its regrowth. This newly quantified cumulative RF arising from ρ_{albedo} , in watts per m^2 and per hectare of a certain forest type, can be combined to regionalised production values for each cover type (in kg/ha) and added to the existing environmental databases (i.e., ecoinvent). From then on out, every attributional analysis using wood products will include the impact of ρ_{albedo} . We hypothesise that the results will be representative of each cover type and will add a predictable influence on the LCA of wood products in Quebec. If validated, the resulting method could be extended to Canada's entire boreal forest. Furthermore, a more comprehensive analysis of the climate impact from non-gaseous radiative agents will lessen the likelihood of selecting sub-optimal solutions. We hypothesise that the results will be sufficiently representative of each cover type to add a predictable influence on the LCA of wood products in Quebec.

5.02.P-Mo261 Environmental Assessment of Lignin-First Integrated Biorefinery Flowsheets: A Comparative Study for Bio-Based Platform Chemical Production

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As the technological improvements in today's world advance, along with the rising demands in consumer products to accommodate the increasing world's population, the demand for platform chemicals has also increased gradually over the past years. For instance, the production of platform chemicals such as phenols have surpassed 10 million metric tons per year in 2015 and is expected to increase to at least 13 million metric tons in 2026. Consequently, climate change phenomena became more and more intensified as greenhouse gas emissions and other polluting substances are increasingly released due to the rise in linear consumption and production patterns. To this end, there are continued efforts to address the multifaceted climate change problem by various strategies, including reducing the fossil fuel dependency for platform chemicals, among others. This study has the ambition to address the significance of this shift towards bio-based platform chemicals rather than the current conventional chemical products derived from fossil fuels. Integrated biorefineries are one of the potential routes for bio-based chemical production, which has significantly lower environmental impacts when compared to conventional fossil-based production. This study investigated the environmental performance of the conceptual novel integrated biorefinery within the scope of the NIBCON project in Flanders, Belgium, based on various process flow diagrams. The integrated biorefinery converted wood chips feedstock into refined lignin oils along with the pulp and the aqueous sugar solution. The refined lignin oil can be subsequently upgraded into platform chemicals like phenols. To this end, a life cycle assessment was performed with the ReCiPe 2016 methodology in order to assess the environmental performance of the conceptual integrated biorefinery, excluding the energy demand of the bioreactor. The obtained results were then compared with the Hock's process, which is the conventional fossil-based production. The results demonstrated that the integrated biorefinery performed better than the conventional process in all cases for all impact categories except for land use and freshwater consumption. The global warming potentials for all biorefinery cases, excluding biogenic carbon, ranged from 0,59 to 2,06 $\text{kg. CO}_2 \text{ eq.}$ for all cases of the integrated biorefinery as opposed to 4,37 $\text{kg. CO}_2 \text{ eq.}$ for the fossil-based production when assessing the environmental performance of 1 kg. of refined lignin oil production. Critical process parameters, such as the energy consumption, water use and solvent recycling percentage, were also identified and investigated by means of the sensitivity analysis to determine further improvements on the integrated biorefinery technology. The research highlighted the importance of limiting or decreasing petroleum-based chemicals dependency, leading to significant reductions in global warming potential. Therefore, the study demonstrated that the novel integrated biorefinery could be an environmentally viable technological concept for producing bio-based platform chemicals.

5.02.P-Mo262 Systemic Assessment of Environmental Impacts of Regional Bioeconomies Using Biogenic By-Products and Wastes - a Stepwise Approach and Selected Results From a CASE Study on a German Metropolitan Area

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By substituting fossil by bio-based resources bioeconomy (BE) concepts aim at being more sustainable while ensuring food and resource security. However, the growing demand for cultivated biomass may increase competition for land and lead to an intensification of agriculture and forestry, contributing to e.g. biodiversity loss. Changing the resource base towards biogenic by-products and wastes and processing them into high value products is part of the circular BE (CBE) concept and discussed as a potential solution to reduce such problems. The transition towards the BE is a global trend, with worldwide more than forty countries implementing political BE strategies. However, the successful implementation of a BE depends on regional conditions. This is especially given in the case of CBEs using biogenic by-products and wastes. Their availability varies regionally due to differences in conditions such as the density and kind of local industry, agriculture, forestry and population. Therefore, it is necessary to evaluate the environmental impacts of CBE at the regional level. In our poster we introduce a stepwise approach to develop BE technology scenarios that reflect regional conditions and to evaluate the environmental consequences of their implementation using LCA. The methodological proposal includes the following steps: (1) regional quantification of biogenic waste and by-products at the NUTS-3 level based on statistical data. (2) Identification of further region-specific conditions (e.g. industry focus, technological know-how) that should be considered in (3) region specific technology scenarios. They comprise a set of biogenic waste and by-product valorising technologies that match the regional conditions. In step (4) the environmental impacts of the selected BE technologies are evaluated using consequential LCA following the need to consider changes in feedstock demand or product supply. Finally, in step (5) the LCA results that are determined at the technological level must be extrapolated to the regional level. Based on the availability of biogenic waste and by-products the mix of technologies that can be

implemented in the region is determined. From the case study on the German metropolitan region Frankfurt Rhine-Main we show exemplary results, including the quantification of biogenic waste and by-products and the environmental impacts from the LCA of an innovative BE technology.

5.02.P-Mo285 Assessing Contribution of Food Label to Biodiversity Through a Predictive Indicator

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The development of an environmental impact label on food products based on Life Cycle Assessment (LCA) is ongoing in France and Europe to meet environmental concerns, especially those linked to biodiversity drastic erosion. LCA shortcomings regarding impacts assessment on biodiversity concern coverage of some biodiversity components, inclusion of some farming or landscape management practices. We present a predictive indicator assessing the contribution of different food labels to biodiversity. This indicator is operational, transparent and adapted to all agricultural productions. Starting from the method of Lindner et al. (2019), we integrated more precise knowledge on the cause-effect relations between practices and impacts on biodiversity into the algorithms, using an original aggregation method based on fuzzy decision tree (Bockstaller et al. 2017). We aimed at describing associated biodiversity and applied the method on food quality and farming management labels (e.g. organic farming). Relevant management variables impacting biodiversity and principles guiding their structuration into a hierarchical decision tree were identified through a literature review. Current state of knowledge guided the CONTRA method parametrization regarding fuzzy class limits, membership functions and decision rules. Data to calculate indicators were retrieved from label guidelines. Missing data due to management items not covered by guidelines were assessed by available observations, or else, by average value from databases or experts. The new indicator distinguishes impacts on, on the one side, mobile species, and on the other side, species considered as “non-mobile” (plot-dependent). Organic farming label yielded good results for the systems we studied (annual crops) while results are variable for other labels and guidelines. In terms of outlook, i) the indicator will be compared to LCA methods and fields observations, for validation ii) calculations may be refined by integration of data on landscape context, and iii) calculation of indicator will be extended to a larger sample of labels.

5.03 Life Cycle Assessment (LCA) in frameworks for policy-making: different scales of assessment, data sources, and policy uses (Part I)

5.03.T-01 Impacts on Fuel Producers and Customers of Conflicting Rules for LCA

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Fuel producers and other commodity suppliers are increasingly affected by conflicting rules for life cycle assessment (LCA). They may get multiple requests for LCAs to be used in various contexts. This may require the application of different methodological approaches that may vary in scope, system boundaries, data demands and more. This results in increased cost and competence requirements for producers as well as confusion among other actors including their customers. Differences in methodologies might also lead to various outcome, conclusions and conflicting guidance regarding what fuel to prioritize or develop. We have studied the actual differences when applying different frameworks. The EU Renewable Energy Directive (RED), the EU framework for Product Environmental Footprints (PEF), and the frameworks of Environmental Product Declarations (EPD) all have different assessment and modelling requirements. The work has contained both an analysis of the methods from a conceptual point of view and by carrying out case studies on selected fuel production pathways (ethanol from corn, fatty acid methyl ester, biogas from food waste, HVO from used cooking oil, advanced ethanol from food waste and sawmill residues and pyrolysis oil from used tires). Results obtained for a specific fuel could differ substantially depending on the framework applied and the assumptions and interpretations made when applying this framework. Especially modelling of waste management can be very important for the results when the biofuel is produced from waste. Our results indicate a much higher climate impact for, for example, HVO and biogas when assessed with the PEF framework compared to the other frameworks. This is because PEF assigns at least part of the production of primary materials and energy to the use of recycled material and recovered energy. Developing Category Rules for biofuels for PEF and EPD could and should help clarifying remaining ambiguities.

5.03.T-02 A Critique of the EU's Revised Renewable Energy Directive and the Delegated Regulation Supplementing the Directive

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The EU's revised renewable energy directive (RED II) and the EU's Delegated Regulation (DR), supplementing the Directive, establish criteria for the determination of high and low indirect Land-Use Change (iLUC) risk associated to the expansion of feedstock production into land with high carbon stock. In this work we argue that the criteria adopted by the RED II and by the DR exclude a-priori some feedstocks due to their country of origin, although they show lower environmental impacts per unit of product and penalizes specific product based on the global direct Land-Use Change (dLUC) associated to them instead of the iLUC. We perform an in-depth review of the RED II, the DR and of the “The Global Biosphere Management Model” (GLOBIOM), which was applied to quantify the LUC GHG emission in two studies commissioned by the EU to support the drafting of the RED II. Based on the review, we highlight the limitation of the RED II and DR especially on the quantification of the iLUC of different sources of biodiesel, in particular: palm oil, rapeseed oil and sunflower oil, and soybean oil. We performed a detailed LCA to compare the GHG emissions of those oil and compared the results with the results obtained with the GLOBIOM model. **1. 2.** The EU's RED II and the DR fail to distinguish between direct and indirect LUC. Instead, they set criteria for

determining iLUC based on the dLUC recorded during the period 2008-2015, although these are entirely different concepts. Thus, the criteria for determining low iLUC-risk feedstock are based on past status and/or past changes. They do not account for the actual iLUC effects triggered when demanding the production capacity of land at a global level. Moreover, the RED II and the DR fail to capture key differences in crop management practices as well as national and regional differences in palm oil production. They fail to distinguish between RSPO certified and non-certified palm oil, even though RSPO certified production explicitly addresses the main sources of GHG emissions occurring in oil palm cultivations: the drainage of peat soil and deforestation. Therefore, they risk compromising the efforts made by RSPO certified palm oil producers in Southeast Asia to reduce the GHG emissions of oil palm, which is the oil crop with the highest yield and thus lowest iLUC per unit of product. We conclude by providing recommendations to correct the criteria for determining high and low iLUC risk feedstock.

5.03.T-03 Considering Spatio-Temporal Dynamics in Assessing Long-Term Scenarios in Territorial LCAs: Application to Irrigated Areas

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Territorial Life Cycle Assessment (T-LCA) is a meso-scale adaptation of the conventional LCA framework, allowing to assess the environmental performance of a territory and an associated land planning scenario while taking into account its multifunctionality (e.g. the economic, social or environmental functions of land use). This framework is used either to diagnose impact hotspots of territories or to compare the performance of territorial land-planning alternatives. In the latter case, eco-efficiencies ratios, i.e. services provided by the territory to its environmental impacts, can be used to identify trade-offs between the socio-economic and environment dimensions. To date, the approach has been static, assessing the impacts of a land use scenario in a given year. However, the environmental performance of a system can be affected by environmental disturbances such as climate change. This work aims at exploring the integration of a dynamic approach in the comparison of eco-efficiencies of territorial trajectories to consider potential environmental feedback in a prospective perspective. The proposed methodological developments will be used to compare the dynamic eco-efficiencies of agricultural planning scenario with or without irrigation considering effects of climate change. Following the T-LCA framework, the scenarios studied were first defined, then, inventory data were collected. In order to take into account the effects of climate change on system performance, the FAO's agronomic crop model AquaCrop is used. It allows considering several effects of climate change such as the increase of temperature and CO₂ concentration, or the change of precipitation regimes on both yields and water needs of crops present in the studied territories. The climate scenario used in this study is the RCP8.5 from the IPCC. The results of this study showed that an environmental feedback on human activity, climate change on agricultural areas, was successfully included in the T-LCA framework. It underlines the fact that it is crucial to consider the spatio-temporal dynamics when performing an environmental assessment to identify false good ideas. Other feedbacks could be considered such as local water depletion or soil erosion to perform a complete prospective T-LCA.

5.03.T-04 Should Human Toxicity of Pesticides Be Considered in Environmental Labelling of Food Products? A Proposal Using LCA for the French Context

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Introduction Environmental labelling of food product is nowadays a key subject for policies and consumers in Europe, and an experimentation is ongoing in France. Its scientific council proposed to base it on LCA and PEF reference framework to assume compatibility with European work. They also questioned the inclusion of residue impacts, pointing that the regulation already ensures the sanitary quality of food with maximum residue limits (MRLs). However, in agri-LCA, (eco-)toxicity is dominated by pesticides and in particular, by ingestion with residues which is the main route of exposure (Fantke and Jolliet 2016). The aim of our research is to highlight the possibility and urgent need to consider pesticide impacts from environmental exposure and residues within LCA for food environmental labelling purpose. **Methods** In Agribalyse, 8 crops representing the main crop families (e.g. cereals) were selected. According to OLCA-Pest recommendations on PestLCI, pesticide emission fractions to the environment and crop leaves were used. Then emissions were allocated to correspondings USEtox_{LC}-Impact and dynamiCROP compartments, respectively to assess environmental exposure and residues. An intermediate approach of LC-Impact with the dynamic version of USEtox 2.12 was developed to have characterisation factors of human non-cancer toxicity at mid-point with a 100 years time horizon. **Results and discussion** Our method allows us to account for the impacts of pesticides including residues on human health in LCA and to compare different types of farming practices. The results show that human non-cancer toxicity is in general 3 orders of magnitude higher than with EF method. Impacts from residues are mostly dominating total human impact of pesticides for conventional productions, except for tubers. Within our 22 scenarios, pesticide residues account for up to 99.5% of all pesticide-related impacts, despite amount of residues estimated to be below the MRLs; hence a potential health impact was assessed. **Conclusion** A methodology to assess human toxicity due to pesticides was proposed for food environmental labelling purposes. We are challenging that MRLs is already wholly addressing human health due to pesticides. Indeed, LCA shows that doses of ingested chemicals below MRLs can still have potential impacts on humans. Thus, environmental labelling of food product should consider impact of pesticides on human health, as recommended by the LCA scientific community.

5.03 Life Cycle Assessment (LCA) in frameworks for policy-making: different scales of assessment, data sources, and policy uses (Part II)

5.03.T-06 A Life Cycle Approach for Sustainable Aviation Biofuels Production: The Carbon Offsetting and Reduction Scheme for International Aviation (CORSA)

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The Carbon Offsetting and Reduction Scheme for International Aviation (CORSA) requires airlines to offset their greenhouse gas (GHG) emissions above 2019 levels either by buying carbon offsets or using Sustainable Aviation Fuels (SAFs). These are defined as drop-in jet fuels derived from biomass or waste. CORSA establishes that SAFs should not be produced at the cost of land classified as primary forests, wetlands or peatlands after 1 January 2008; and have at least 10% lower life cycle GHG emissions than conventional kerosene. GHG reductions shall be calculated by using life cycle assessment (LCA), including emissions from direct land use change (DLUC). This study estimates potential GHG savings of SAF production pathways, considering variability in DLUC emissions. Attributional LCA is applied to quantify gCO₂-eq/MJ from well to wake; combined with IPCC's Tier 1 approach. Several oilseeds, as well as lignocellulosic and starch-based crops, are examined as feedstocks to be respectively used in Hydroprocessed Esters and Fatty Acids (HEFA) and Alcohol-to-Jet (ATJ) pathways. Changes in GHG emissions relative to fossil kerosene vary widely depending not only on the feedstock but also on the underlying land use transitions and crop management practices. Lignocellulosic and starch-based crops deliver substantial GHG savings, except in scenarios with conversion of improved grassland (for maize and switchgrass only) or temperate forests. Camelina, pennycress, and salicornia offer GHG advantages over other oilseeds, due to assumptions on co-products' allocation. Jatropha and oil palm show a better GHG performance than annual oilseed crops, mainly due to soil organic carbon gains and carbon sequestration in crop biomass. Both perennial crops meet the CORSA threshold unless produced at the cost of carbon-rich land uses. Soybean, rapeseed, castor bean, and tobacco may only be eligible for HEFA production if grown on arable land or degraded grassland. Results highlight how variability in DLUC is critical in determining the GHG implications of SAFs. DLUC emissions can negate any potential GHG savings of both HEFA and ATJ pathways, especially when forests or natural shrublands are lost, which justifies the need for the CORSA sustainability criteria. The adequate consideration of DLUC effects becomes crucial for the future development and application of CORSA, to ensure the deployment of SAF technologies and effectively promote carbon-neutral growth.

5.03.T-07 Assessing a Project Environmental Footprint: A Methodological Framework

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There is currently a need for comprehensive, holistic and multi-criteria assessment methods to evaluate the environmental impacts of a project and to support decision-making. "Project Footprint" (Empreinte Projet in French) is an operational framework, developed by the French Agency for Ecological Transition (ADEME) to assess any project environmental footprint. Its aim is to help project leaders and stakeholders to assess a project or an action. The Project Footprint methodology proposes an interpretation of consequential LCA for the projects whose implementation does not lead to large-scale changes in the economic system, by considering the direct and indirect consequences of a project. It includes the contributions of ADEME "QuantisGES" method and make it more robust with a multi-criteria approach and requirements inspired by international LCA standards. In this method, the project footprint is calculated as the difference between the impacts of a reference scenario with no action taken and the impacts of a scenario where the project occurs. The Project Footprint methodology offers five maturity levels, from the simple qualitative assessment to a structured quantitative assessment that can be communicated externally. As an operational approach, it guides the user all along its application of the methodology: It gives guidelines and a step-by-step method which starts with a characterisation of the targeted project, to structure a consequence tree and to calculate the quantified project's impacts. Furthermore, the methodology also offers some support to conclude on the project environmental relevance with a level of reliability. Additional recommendations are made regarding the results communication, according to the selected approach level. To facilitate the application of the methodology, real-life case studies are provided to the user. The Project Footprint methodology is a first step to assess a project's environmental impacts by providing a transparent and reliable framework. In order to help better decision making, the methodology can be adapted to the context and objectives of the assessment and gives the user relevant environmental information.

5.03.T-08 Towards a Simplified Life Cycle Assessment Tool for Urban Agriculture

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Urban agriculture (UA) is emerging as a tool for cities to tackle multiple social and environmental issues. As cities and regions support the development of UA through dedicated programs, food planning, and urban policies, questions remain about its environmental impacts. Life cycle assessment (LCA) is useful to quantify impacts, compare types of UA, and assess the actual benefits (or impacts) that are assumed to come with UA. Our goal was to create a simplified LCA tool adapted to UA to support policy makers and planners. This would help them decide which types of UA to implement, quantify the contributions of UA to urban sustainability goals, and could lead to product-level impacts for communicating with consumers. Through a systematic literature review and meta-analysis, and work with 9 UA case studies, we advanced and questioned several features of UA LCAs. A main takeaway was that very little is known about the environmental performance of UA, and relatively few LCAs have been done. Therefore, there were not enough resources or knowledge to simplify LCAs for UA. Main barriers were the variability of UA and uncertainty about UA practices. Indeed, from our work with diverse case studies, we witnessed great difficulties in data collection due to changing practices, the young age of farms, regular turnover of farmers and volunteers, and lacking focus/interest in data collection because of other main goals. In a first step to address this, we created an LCA framework and set

of guidelines to improve and harmonize UA LCAs, discussing system boundaries, data sources, and system modeling decisions. More data area needed about practices in UA, so we provided recommendations for how to collect this data, and summarized useful values for when collection is not possible. This framework is the first focused attempt at making UA LCAs more accessible, feasible, reliable, and consistent. Our findings can directly support the creation of a simplified UA LCA tool in a next step, and an improved body of literature on UA LCAs at the farm-level. Better farm-level LCAs will improve studies of scaled-up UA at the city-level (or beyond to the national-level) to evaluate, for example, how UA could help meet cities' climate change reduction goals. We concluded that although LCA of UA may not yet be mature enough for tools or streamlining, assessments of individual case studies can yield valuable insight for improved design and management of UA.

5.03.T-09 Technology Choice Model As an Option to Support Decision-Making in Policy-Making: A CASE Study on Allocations in the Petrochemical Industry

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Standard LCA studies with one on one comparisons can support policy-making, but often a greater context is relevant to guide policy-makers. This is especially true for interlinked industries such as the petro-chemical industry where most processes produce multiple products simultaneously (multifunctional processes). The Technology Choice Model (TCM) can be one option to support decision making. TCM allows decision making in a broader context while still being able to represent production alternatives at the technological level, and consider supply chains and their alternatives. We use a case study on the chemical industry with a focus on the influence of allocations on decisions. Allocation methods are common practice to split environmental impacts between products of a multifunctional process. Our case study covers 24 bulk chemicals and 293 production technologies based on naphtha and natural gas. Products include methanol, ethylene, propylene aromatic compounds and their derivatives. All technologies are described by mass and energy balances, which are used to build a technology matrix. Results demonstrate that decisions without massive use of allocations are possible and usage of allocated data lead to wrong decisions. Additional emissions due to the usage of allocated data vary between 20% and 160% in our case study depending on the allocation method and compared to a system expansion approach. A comparable system could also be used to assess policy measures and their effect on supply chains or the introduction of emerging technologies, such as bio-chemicals. Further applications could include recycling intense industry such as plastic or metal industries and assessment could be expanded for multi objective optimization.

5.03.T-10 Sketching Science-Based Environmental Transition Plans for Industries With EEIO: Illustration by Canada's Road SECTOR

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Building effective environmental transition plans for industries and companies is challenging due to the lack of quantitative understanding of their ecological responsibility over their value chain. Companies mostly turn to Life Cycle Assessment (LCA) to perform specific environmental quantifications. Yet, Environmentally-Extended Input-Output (EEIO) is very efficient on a wider scale. We illustrate the usefulness of EEIO in the case of the Canadian road industry. An EEIO database has been developed for Canada: Open-IO Canada. The database uses different sources of inventories: economic supply and use tables as well as GHG emissions and water use reported by StatCAN, the governmental body responsible for Canada's statistics, and pollutants reported by Canadian facilities in the National Pollutant Release Inventory. Open-IO Canada then uses ImpactWORLD+ characterization factors to estimate the multicriteria environmental impacts of economic sectors or products and services. This database is used to understand key environmental drivers of Canada and its road sector with a consumption-based approach. Results show that the construction sector is the second most impacting sector in Canada (10-31% depending on the indicator) but that the road industry impacts are more limited (0.6-1.8%), with 1.3% of GHG emissions and damage to human health and ecosystems, mainly driven by bridge materials and energy consumption. Priorities could be to reduce the impact of concrete and crude oil purchases, and direct emissions through the investment in new machinery. The contribution of activities often neglected in roads LCA (capital goods, staff consumptions, services) is substantial. Direct emissions present very low contributions except for the GWP (29%): focussing environmental strategies on scope 1 has a limited impact if not done by the entire supply chain. Good correlations ($R^2 > 0.9$) of intermediate purchase contributions between GWP and the two damages point out GWP as a suitable proxy for holistic evaluation of this industry's green purchase plans. Finally, on the life cycle of Canadian roads, construction and maintenance account for 5% of GHG emissions, against 67% for its use stage and 27% for vehicle manufacturing and maintenance: focusing on the road industry is not a priority to decarbonize road transportation, contrary to building and maintaining roads to lower vehicle consumption and wear, or directly reducing vehicle manufacturing and tailpipe GHG emissions.

5.03 Life Cycle Assessment (LCA) in frameworks for policy-making: different scales of assessment, data sources, and policy uses (Virtual Only)

5.03.V-01 Advanced Wastewater Treatment Technologies: What Are the Techno-Economic and Environmental Challenges?

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Surface water quality is threatened by the release of organic micropollutants (OMP) originating from human activities. Policy makers have different options to counteract this trend, one of them is the upgrade of existing wastewater treatment plants with advanced treatment stages, capable to remove or degrade OMP. Switzerland is the first country which has implemented a

nationwide legislation requiring wastewater treatment plants (WWTP) to reach an 80% removal from a set of indicator substances. On the EU level however, there is still a controversial discussion on how to evaluate advanced treatments, considering technoeconomic and environmental aspects. With this contribution, we want to fuel the discussion on the base of a comprehensive summary of the technological state of the art of advanced wastewater treatment options. Our systematic search shows that conclusive life cycle assessment studies are still underrepresented in the peer-reviewed literature compared to the available technological data, but highly needed for implementing sustainable solutions. We concluded that all implemented treatments have strengths and drawbacks. For example, while the removal efficiency of membrane processes is outstanding compared to adsorption or (advanced) oxidation processes, high energy demands make their application unattractive in regions with elevated energy prices, and sustainability will depend on the energy mix used. In Europe, there is a trend to implement activated carbon adsorption and ozonation. Despite the availability of full-scale data, the reviewed LCA studies came to contradictory conclusions on their environmental impact. This shows that the individual selection of system boundaries and regionalization of impact categories can improve the case-by-case evaluation but on the expense of a decreased transversal applicability of the studies. The lack of data regarding novel advanced treatment technologies, especially in the field of photocatalysis and electrochemical treatments forces researchers to base environmental analysis on lab-scale data, provoking a paradigm shift towards prospective LCA.

5.03.V-02 Comparing Data Sources for National Consumption Footprints: The CASE of Germany

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Policy efforts increasingly focus on the environmental impacts of production and consumption along the full supply chain of products. For this purpose, the European Commission – Joint Research Centre (JRC) developed the Consumption Footprint, a Life Cycle Assessment (LCA)-based framework that assesses the environmental impacts of EU consumption through a full bottom-up approach based on around 160 representative products in five areas of consumption (i.e., food, housing, mobility, household goods and appliances). This indicator could be implemented by EU countries such as Germany to complement existing monitoring frameworks. However, the Consumption Footprint targets the implementation in all EU member countries and, therefore, employs EU-wide national-level data sources (such as Eurostat), while there are additional national data sources that could be used for a more fine-grained analysis. The goal of this study is to compare the use of different national data sources for the assessment of the environmental impacts of German consumption using the approach of the JRC Consumption Footprint indicator. The exercise followed the three-step process of the Consumption Footprint with specific focus on the calculation of the consumption intensities, where the two different data sources were compared. The scope of the analysis covered the consumption of (average) German citizens for the time period 2010-2018. Several national data sources could be employed for the analysis, requiring a clear prioritization, and data gap filling. Comparing data sources and resulting impacts unveiled different levels of detail and of scope. On the one hand, for some areas of consumption and product groups, more detailed data sources are available at national level (e.g., from consumer spending surveys). This can support capturing emerging consumer product trends (e.g., increased use of e-bikes or tablet PCs) and highlight missing products. On the other hand, consumption intensities can vary due to different scopes (e.g., focusing on household vs. entire consumption; inclusion of indirect consumption in services). The study concludes by comparing results obtained with the Consumption Footprint approach with national footprint estimates and downscaled planetary boundaries for Germany. Finally, ideas on how the indicator might complement existing monitoring frameworks are highlighted.

5.03.V-03 Coupling Agent-Based Modelling With Territorial LCA to Support Agricultural Land-Use Planning

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The development of the life cycle assessment (LCA) adapted at the larger scale - “territorial LCA” (TLCA) - shows advantages in assessing the environmental impacts of a multi-functional territory. However, the TLCA approach and its developments are limited to evaluating the impacts of the land-use scenarios on a static basis. In contrast, a territory is a complex and dynamic system in which individual decisions play a significant role. This work aims to develop a dynamic territorial LCA approach linking TLCA with agent-based modelling (ABM), a widely used tool to simulate individual agents’ decisions for supporting policy analysis and planning. A case study was implemented to examine the bioenergy crop development with demonstration farms installed at different locations in an agricultural territory (Wallonia). We consider farm size, revenue, and familiarity (distance to successful adopters) as influential factors. Geographical Information Systems (GIS) was employed for spatial data treatment. The results show that different initial locations for demonstration farms would lead to different dynamic results at the territorial level. The case study presents the capability of spatio-temporal simulation of the territory dynamics with the developed model, taking into account farmers’ adoption decisions and site-specific conditions. Such knowledge on the interactions between human behaviours and land-use scenarios could bring insights for policy-making, supporting reasonable land planning at the territorial level. Besides, by employing GIS to estimate spatially explicit inventory of unit processes, this work fills the research gap of the lack of application linking LCA, ABM, and GIS together. While the case study was conducted for bioenergy crop development, the coupled framework is by no means limited to it, and other areas such as forest management may benefit from the framework. Furthermore, this work will benefit from cutting-edge research such as life cycle sustainability assessment, behavioural economics, and social networks.

5.03.V-04 Critical Transmission Industries in the Global Supply Chain Networks to Reduce Embodied Emissions

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Productions and consumptions of goods and services have clearly contributed to the economic growth in the world, whereas those economic activities brought about environmental loads, especially greenhouse gases (GHG) emissions. Industries and firms seek to reduce their environmental loads of their products through their whole supply chains, that is, *environmental footprint* (e.g., carbon footprint) to contribute the climate change mitigation. Therefore, supply chain management from upstream to downstream industries i.e., the inter-industry (or inter-firm) collaboration are essential in order to reduce the supply chain emissions effectively. In the field of industrial ecology, the environmentally extended input-output analysis has been used to trace the embodied GHG emissions in whole supply chains. The set of industries and the inter-industrial transactions in the input-output table can be regarded as *network*. Here, we apply the concept of *betweenness* originated from network analysis to the input-output analysis, and develop the new approach to identify transmission industries environmentally critical for supply chain management. In the perspective of global economy, we especially focus on the territorial emissions embodied in international exports to utilize the demands for both final and intermediate goods in foreign countries. Critical transmission industries here represent the hub industries which participate in the global supply chains and induce domestic territorial emissions. These industries have potential to enhance the creation of domestic value-added from global supply chains and simultaneously contribute emission reduction targets such as Paris agreement. We use Eora, the global multi-region input-output table, and identify the environmentally critical transmission industries with a focus of the industries in Japan, United States and China as a case study. This new approach can help firms to find opportunities to mitigate GHG emissions in their supply chain networks.

5.03.V-05 Increased Ecotoxicological Impacts Due to Reduced Pesticide Application: An Anomaly in Designing Low-Input Paddy Rice Production and Necessity of Precise Characterization

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Pest management strategies to improve agricultural sustainability aim to minimize human health and environmental risks associated with pesticide use. However, some management practices, such as a low-input agricultural system that reduces the pesticide use by adjusting the active ingredients (AIs), may induce the use of low-dose alternative AIs with a higher efficacy on target organisms but at the same time a stronger ecotoxicological impact on non-target organisms, consequently leading to an unintended increase in risk. Here we developed a precise evaluation method that can better distinguish the differences among AIs and designed an impact assessment of pesticide uses on eight paddy farms in Japan using the method to demonstrate the effectiveness of a transition from conventional to low-input managements. The proposed precise method contains a simulation model to calculate pesticides emitted from a paddy field, and a list of characterization factors (CF) originally calculated based on a nonlinear concentration-response function (CRF). This allows providing detailed emission results considering local water management in paddy field and precise CFs considering parameters both the hazardous concentration potentially affecting 50% of the species (HC50) derived from a species sensitivity distribution (SSD), and the slope of the SSD, and hence can lead to a better understanding of the effects derived from the change in AIs. The results of the case study showed that, in general, the reduction in the number of AIs applied contributes to an impact reduction, but the effects varied widely on individual farms, especially with some farms (three of the eight farms) resulting in an opposite increase in impact. The factors causing the increased impact are found to be not only the use of a highly toxic alternative substance, but also the change in emissions. The proposed precise method was confirmed can better capture the difference among active ingredients, and consequently, effectively contribute to the farm's impact reduction goals due to pesticide use.

5.03.V-06 The Impact From LCA on Policies for Plastic Waste Handling in the Fishing and Aquaculture SECTOR

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In this paper, we identify how LCA and life cycle perspectives could help to create a circular economic value chains in accordance with sustainability for plastic waste handling in the fishing and aquaculture sector. Our research questions are: (1) What existing (regional), national and European policies directly and indirectly affect handling of plastics in the fisheries and aquaculture sector? (2) How can new policies based on LCA and life cycle perspectives promote more sustainability and circularity for handling plastics in the sector? Thus, addressing how LCA in the future could be used to improve policy in a more environmental benign direction in the fisheries and aquaculture industry.

5.03 Life Cycle Assessment (LCA) in frameworks for policy-making: different scales of assessment, data sources, and policy uses (Poster)

5.03.P-Tu288 A Life Cycle Assessment Framework for Improving Household Refrigerators Sustainability of Food Systems: A Review

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Food waste (FW) leads to substantial economic, energetic, and environmental loss worldwide. According to Eurostat's Domestic Material Consumption (DMC), the food sector requires a total of 1.44 Gt material resource input per year, which accounts for 20% of the European Union's total DMC. It is estimated that 35% - 62% of avoidable FW occurred at the household level in developed countries, reflecting great potential opportunities for reducing environmental and social impacts. This review focuses on embodied environmental trade-offs between household refrigerators and the food system associated with FW at a consumer level. A well-integrated household refrigerator with a food system benefit for community's health, food security, and nutrition. This study mainly explores the inherent trade-off of reducing FW and its associated environmental impacts by deploying

household refrigerators, a refrigeration technology that would increase environmental burdens due to energy consumption and refrigerant leakage emissions. Therefore, it examines the trade-offs between the increased energy use due to food refrigeration and the GHG savings of reduced FW at a household level.

5.03.P-Tu289 Freshwater Ecotoxicity Assessment of Measures Promoting Agricultural Biomass Production Can Inform Policy Making on Biofuels

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Sweden's ambitious climate-mitigation strategy for the transport sector is dependent on the possibility to increase its agricultural biomass production for biofuels. Policy stimulating agricultural biomass mobilization is needed to keep pace with a high biofuel implementation rate, but large-scale agricultural production changes pose a series of environmental risks. Assessing the environmental impacts of policy alternatives for promoting agricultural biofuels is therefore a much needed scientific input for policy-making. However, effectively quantifying the environmental impacts of policy measures is challenging due to the dynamic nature of agricultural systems, which involve complex interactions among human actors and the environment. This study combines an Agent-Based Model (ABM) with Life Cycle Assessment (LCA) methods, and aims to assist decision-making to quantify the potential ecotoxicity implications of relevant agricultural policy alternatives. First, we model spatially-explicit production shifts likely to occur in an intensive arable cropping region in Sweden if farmers are required to grow a minimum proportion of their farm area as grass ley. This is done by means of AgriPoliS, an ABM that is frequently used to predict farmers' responses to changes in agricultural policy. The novelty of our study is that we then couple the ABM results to LCA methods to expand the policy analysis to a relevant environmental impact category. This is done by assessing the potential freshwater ecotoxicity impact of predicted changes in pesticide application by means of the chemical characterisation model USEtox and pesticide fate model PestLCI, a site-specific pesticide inventory and local soil properties and climate. While literature suggests that lower returns from grasses can be compensated over time with higher productivity due to soil benefits of leys in crop rotations, our results show the extent to which mobilizing biomass with low farming inputs in intensive farming regions can reduce impacts from pesticide application. Our approach therefore establishes a quantitative relationship between policy promoting biomass and its implications in terms of regional freshwater ecotoxicity impacts. This contributes to expanding the environmental assessment of land-based biofuels at a time when their overall sustainability is highly debated.

5.03.P-Tu290 Environmental Assessment: ADEME Guidance to Select the Right Methods

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Expectations in terms of environmental assessment (EA) have risen considerably during the last decades, while at the same time the methods have become more sophisticated and diversified to meet needs that have gained in maturity. There are thus many EA methods, which may be more or less related, addressing different contexts, scope of evaluation, periods or even assessment objectives. EA must also adapt to various users and prescribers. All this emphasizes the importance of having a common framework allowing for harmonized implementation of EA. The goal of the guide presented here is to serve as a basis for such a framework. We believe anyone interested in EA can find this guide useful to better understand the role and limits of the main existing methods, and which one to select depending on one's context and objectives. This guide aims to help understand the various EA methods and to choose one or more methods adapted to a given assessment context. It contains three main complementary components: 1. Twenty individual factsheets allowing, in a few pages, to become more familiar with a given method. 2. A mapping of these methods in order to better understand their respective positioning; 3. A decision-making flowchart to help users to select the method(s) adapted to their needs. The purpose of this guide is to help anyone select one or more methods suited to its needs in terms of Environmental Assessment. However, it is only a first step towards a better understanding of EA. It does not deal with the operational and detailed implementation of each of the methods investigated, and it could and should be supplemented by specific frameworks, training modules, guides of best practices and so on. ADEME is currently working on it and welcomes cooperations.

5.03.P-Tu291 Environmental and Social Impacts Assessment Caused by the Growing Demand for Electric Vehicles

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Of the global greenhouse gas emissions, transportation CO₂ from gasoline and diesel combustion from all forms of road traffic, including cars, trucks, and buses, accounts for about 16%, of which passenger travel accounts for 60% of the total [1]. In addition, the LCA (Life Cycle Assessment) of EVs shows that the environmental impact up to the manufacturing stage is significant [2]. It has been predicted that the stock of EVs will reach 230 million units by 2030 [4]. The stock of EVs is expected to reach 230 million units by 2030 [4], and restrictions on gasoline and diesel vehicles are scheduled to be imposed by 2030, mainly in Europe. The increasing demand for each component due to the shift to EVs is expected to have a significant impact on the raw material procurement stage. Rare metals are indispensable in the current EV manufacturing technology, but the use of rare metals poses a great risk. In particular, cobalt which is used in lithium-ion batteries is heavily sourced from the Democratic Republic of Congo (DRC), where social risks such as child labor and work hazards have been reported [5]. In addition, there are concerns that the shift to EVs may not only lead to more serious social risks but also the loss of natural capital associated with mining. Thus, the increased demand for rare metals used in batteries can contribute to the deterioration of natural, human, and social capital, but there is no method to properly assess these impacts. The purpose of this study is to examine the impact of the shift to EVs on climate change and the associated social risks using the LCA methodology. Conduct an inventory analysis in accordance with ISO 14040, the international standard for LCA. The social impact will also be calculated. For the meta-analysis of LCA studies, we will collect automotive LCA studies that have been conducted so far and extract process data on raw material production, assembly, use, and recovery/recycling for each vehicle model, or quote data from integrated reports. These data will be analyzed

using LCA software and the inventory results will be discussed. If any missing data is identified in the cited data, it will be supplemented with a substitute database or alternative values. As for social impacts, we will use the Social Hotspots Database and A Product Social Impact Life Cycle Assessment database to calculate and discuss the impacts on child labor, forced labor, and gender equality. Show the number of hours worked under each impact, compare by vehicle type, and explore where the supply chain hotspots for specific EVs exist. [1] Our World in Data, *Sector by sector; where do global greenhouse gas emissions come from?* Hannah Ritchie, September 2020 [2] Goldman Sachs, *New era in Co2 regulation: EVs to be tested across life cycle, not only on running performance*, December 2019 [3] EUROPEAN COMMISSION, *'Fit for 55': delivering the EU's 2030 Climate Target on the way to climate neutrality*, July 2021 [4] IEA *Global EV Outlook 2021*, April 2021 [5] Amnesty International, *"THIS IS WHAT WE DIE FOR"*, 2016

5.03.P-Tu292 Should We Use Plastic Shelters to Protect Seedlings for Afforestation?

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Restoration of forested land represents an effective strategy to contribute to net-zero target emissions by enhancing the removal of greenhouse gases from the atmosphere. The most common afforestation strategy envisages planting seedlings, which are germinated and grown to the desired age at tree nurseries, with plastic shelters to increase growth and survival of trees. However, the use of plastic shelters is hotly debated, not least because shelters are often left on-site, contributing to plastic pollution in the environment. This work presents a comprehensive Life Cycle Assessment (LCA) study that compares the environmental impacts of current and prospective scenarios for plastic shelter-aided seedling planting compared with a base case where shelters are not employed. The underlying objective is to support policy- and decision-making. The work focuses on the UK but results and conclusions are valid for other temperate oceanic regions. The scenarios investigated are a combination of different shelters materials (including bio-based ones) and end-of-life strategies. The analysis demonstrates that (i) on average planting seedling without shelters is the most preferable option across most environmental impact categories (including Climate Change), and in terms of weighted results, (ii) polypropylene shelters are preferable to bio-based alternatives, including polylactic acid-starch blends and bio-polypropylene, (iii) recycling is the most environmentally advantageous end-of-life treatment. The LCA results also show that the carbon emissions of the scenarios investigated are negligible when compared to the amount of carbon sequestered by a tree in 25 years.

5.03.P-Tu293 Developing Policy Recommendations for Short Food Supply Chains in Europe

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Food production in many areas is distant from consumers, both spatially and socially, and value creation is concentrated in a few actors with high market power. Moreover, food supply contributes significantly to climate change (GWP) and other negative environmental impacts such as eutrophication and acidification. Short food supply chains (SFSCs) can potentially contribute to reducing these problems and are therefore an element of the EU's rural development strategy in the context of the Common Agricultural Policy. This raises the question of what characteristics SFSCs need to have in order to promote better sustainability. The integrated sustainability assessment of selected case studies in the presented project therefore consisted of a sustainability assessment of SFSCs in comparison with conventional food chains. We present exemplary results for GWP for one type of SFSCs (cooperative shop) compared to the conventional supply chain in the rural (RSC) and the urban (USC) context. Transport distance, type of vehicle for consumer transport and quantity of products purchased by the consumer for a given purchase act could be identified as the key parameters for the overall GWP of a food chain. For the SFSC (electric vehicle charged with low emission electricity) to have a lower impact than the RSC, the food basket needs to be of at least 8kg. For the USC, the food basket needs to be of at least 16 kg. In order to derive recommendations for decision-makers, it was necessary to generalise the case studies to a higher level and to calculate scenarios derived from them. Due to the relatively high uncertainty, we did consider different sensitivities for most relevant aspects in order to obtain more robust results and recommendations. Through explicit consideration of the value chain, the recommendations can be derived for each actor (consumer, producer, policy-maker). For the policy-makers, we came to the following main points: Firstly, to foster the development and continuation of traditional farmers' markets and cooperative shops, where primary producer can sell their goods in an easily accessible public area – ideally with complementary shops nearby. Secondly, to provide platforms for primary producers to connect and build networks of complementary primary producers. Implementing these recommendations will enable consumers to minimize their individual transport per complete food basket, thus reduce the specific environmental impacts of shopping trips.

5.03.P-Tu294 Pathways to Planetary Health Diets: Are National and Regional Food Based-Dietary Guidelines in Line With the Environmental Targets of Eat-Lancet Recommendations?

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Dietary choices are shifting globally - driven by social and technological breakthroughs- in ways that are negatively affect both human health and the environment. A sustainable transformation of dietary patterns and the food systems that deliver them is an urgent need in order to meet the Sustainable Development Goals and the Paris Climate Agreement. The convergence of global dietary patterns around predominantly plant-based diets is a global challenge, mainly due to the substantial impact of the livestock sector on water quality and greenhouse gases emissions. Given the severity of the problem and their multinational nature, global

public health dietary recommendations should complement national and regional government food-related initiatives, such as national food-based dietary guidelines (FBDGs). With these needs and challenges in mind, the EAT-Lancet Commission developed a global planetary health diet, which consists largely of a diversity of plant-based foods and low amounts of animal source foods. Therefore, the purpose of this research was to compare the global EAT-Lancet recommendations with dietary guidelines from Northern and Southern Europe and America in terms of carbon footprint (CF) and water footprint (WF). This study employs a Life Cycle Assessment methodology to enable a complete evaluation of the CF and WF. The individual recommended daily dietary intake was chosen as the functional unit. The analysis was carried out from a cradle-to-consumer approach and the system was divided into three stages: i) Food production stage, ii) Distribution to wholesale and retail and iii) Distribution from retailers to households. According to the results, when compared EAT-Lancet to other recommended diets in Europe and America, none met all the environmental targets set by EAT-Lancet in all food categories. Overall, negative deviations around CF and WF using EAT-Lancet as a reference were higher for dairy. Therefore, moderate dairy reductions in national and regional FBDGs have a significant potential for transitioning to low carbon and low water footprint eating habits worldwide. To sum up, this work identifies that shifts towards universally sustainable diets could generate collateral benefits, such as minimizing diet-related GHG emissions and the protection of water resources to reduce the environmental footprint associated with food consumption, as well as promoting habits that improve the health of the population.

5.03.P-Tu295 Environmental Assessment of Introducing Lupin Crop in a Wheat-Base Rotation System Under an Ecological Regime

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The diversification of agricultural activities is a fundamental strategy to face soil degradation, food security, and climate change. In this sense, the incorporation of legumes in cereal-based rotation systems is presented as a sustainable strategy to improve soil nutrient levels and reduce environmental impacts. This work aims to evaluate, from an environmental perspective, the effect of lupin cultivation in a Galician winter wheat-based rotation system under an ecological regimen. The life cycle assessment (LCA) methodology was applied for a rotation system over a six-year period, which considers Galician winter wheat as the main product, and lupin and potato as the alternative products. Therefore, the rotation system evaluated (RS1) has the following cultivation sequence: lupin (LPN) + Galician wheat (Ge-WW) + potato (PT). The overview of this study was to collect primary data associated with the rotation crops to quantify their environmental impacts, identifying their advantages or disadvantages. Moreover, the system under study is compared with the rotation system without lupin cultivation (RS2), i.e., Ge-WW + PT. Comparing the environmental profiles of both rotation systems was based on a land management approach as the functional unit (i.e., in terms of ha). In addition, the impact categories evaluated are Global Warming (GW), Freshwater eutrophication (FE), Marine eutrophication (ME), Terrestrial ecotoxicity (TET), Freshwater ecotoxicity (FET), and Fossil resource scarcity (FRS), through the Recipe v1.04 midpoint method. Some of the results indicate that in the GW category is obtained 3.07 t CO₂e, meanwhile, 3.42 kg P eq in FE, 38.12 kg N eq in ME, and 633.03 kg oil eq. Moreover, comparing both rotation systems, in the GW category the introduction of lupin led to an impact reduction of 25%. In FE produce an increase of 29%, meanwhile in ME leads to a reduction of 80%. Regarding eco-toxicity-related categories, include lupin led to a slight increase in both with about 1% and 10% for TET and FET, respectively. Finally, FRS also presents a marginal increase of 2%. This study encourages stakeholders and decision-makers to understand the environmental impacts of diversification strategies in order to achieve sustainable agricultural systems.

5.04 Life Cycle Impact Assessment modeling and application (Part I)

5.04.T-01 Fate Factors for Microplastics Emitted in the Marine Environment

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Microplastics (MPs) impacts in the marine environment have been linked to their physiology. Because of their small size (1 μm–5mm), they are available to the marine ecosystem through ingestion, egestion, and tissue transfer. Impacts have been linked to growth hindering, blockage of the digestion tract, internal and external wounds, and even mortality. Since MPs emissions are expected to keep on increasing, assessing their potential impacts in the marine environment is important. Current life cycle assessment (LCA) does not account for MPs. Life Cycle Impact Assessment (LCIA) does not provide characterization factors (CFs) to quantify potential impacts of MPs emissions to the marine environment, which is underestimating the potential impacts of the products studied. MariLCA (Marine impacts in LCA) is an international working group supported by the Life Cycle Initiative and FSLCI. It focuses on developing harmonized CFs for environmental impacts pathways of marine litter, especially plastics. This project falls within MariLCA's work, by developing FFs for MPs emitted to oceans. FFs are developed for different marine sub-compartments (beach, water surface, water column, and sediments) at two different scales (continental and global), identified in a recently developed framework, since the fate mechanisms and the exposure of the marine ecosystem to MPs differ within the ocean. This study prioritizes main parameters influencing the fate in the marine environment leading to the development of FFs for different MPs categorized based on their physical properties, such as shape (cylinder and spherical) and density (low, intermediate, and high). Moreover, this work identifies the need for regionalization based on the influence of environmental factors (water currents, biofilm density, etc.) on the fate. For each category, a rate *k* matrix is developed that accounts for rate transfers of MPs between different marine sub-compartments, and removal rates (including degradation, and deep burial). Transfer rates are obtained using a tracking model (TrackMPD) that records the trajectory of virtual MPs emitted at different locations in the oceans. FFs are then obtained from these *k* matrices. They are expressed in kg per $k_{emitted}/d$. CFs can then

be obtained by combining FFs with exposure-effect factors for micro/nanoplastics potential physical impacts on the aquatic environment (marine and freshwater).

5.04.T-02 Effect Factor (EF) for Macroplastic Debris Entanglement: A Field-Based Species Sensitivity Distribution (SSD)

Approach

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The input of non-degradable plastic waste to the natural environment is projected to further increase and the harmful impacts that it has on marine species are well known. However, there exists no operational methodology in life cycle impact assessments (LCIA) that accounts for this side effect of mismanaged plastic products. As the marine environment in general is underrepresented in the LCA framework, advancing novel impact assessment models pertaining marine ecosystems is instrumental in making the analyses more comprehensive. One of the most well documented ways through which plastic debris may affect marine fauna is entanglement, but a global overview of the impacts of this stressor is missing. Estimates of global plastic debris concentrations are not sufficient as proxies for impact, as the mere presence of plastic debris does not imply that all present species will be affected. As such, a potential effect factor (EF) should be based on estimates of entanglement prevalence, to account for interspecific differences in sensitivity to this stressor. By creating a field-based species sensitivity distribution (SSD) with entanglement rates gathered from 20 species, we model the potentially affected fraction (PAF) of species globally, and present a spatially explicit EF. The predicted EF was found to vary globally from 1.8×10^{-3} to 2.9×10^{-1} PAF/kg.km⁻². The modelling approach implies that the size of the effect following an input of plastic debris is smaller in areas with an already high fraction of species potentially affected. As such, known plastic debris hotspots such as oceanic gyres, marginal seas and coastlines were linked to a lower EF value. However, only when the EF is combined with an appropriate fate factor (FF) predicting the distribution of a plastic debris emission, the spatially explicit impacts can be understood in the form of characterization factors (CF). The presented EF is thus a first step in the derivation of a complete CF model. The current work sheds light on the entanglement potential of plastic debris, and can allow for a more valid evaluation of environmental impacts linked to plastic production, usage and subsequent deposition if applied in the LCA framework. Moreover, as LCA is a widely used environmental management methodology, including this impact can contribute towards more sustainable marine ecosystem management.

5.04.T-03 Impact Assessment of Microplastic Emissions in LCA: Preliminary Characterization Factors for Expanded Polystyrene and Tire and Road Wear Particles

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Although life cycle assessment (LCA) includes a variety of potential environmental impacts (climate change, ecotoxicity, etc.), to date there is no LCA methodology to include the potential impacts of plastic litter. This limits the applicability of LCA as a tool to compare potential impacts of single-use plastics and their alternatives. To address this shortcoming of LCA, the international scientific work group MarILCA (MARine Impacts in LCA) was founded in 2018 with the goal to propose a methodology for assessing (macro-, micro, nano-) plastic litter impacts (marilca.org). Within the MarILCA framework, the new impact category *physical effects on biota* addresses impacts associated with physical effects of plastics such as entanglement (of macroplastics) or ingestion (of microplastics), which ultimately may affect ecosystem quality. This work proposes two examples of preliminary characterization factors (CFs) for assessing potential impacts of microplastic emissions in aquatic environments via the impact category *physical effects on biota*. To develop these CFs, effect factors for assessing the potential impacts of micro- and nanoplastics on aquatic environments proposed by Lavoie et al. (2021) were used. To complete the preliminary CF modelling, simplified fate factors are developed for two types of microplastics: expanded polystyrene (EPS) and tire and road wear particles (TRWP). This exploratory work aims at understanding the relevance of different transport and removal mechanisms influencing the fate of microplastics in aquatic environment, identify challenges and guide research efforts. For EPS and TRWP, this work tested different fate scenarios for the degradation and sedimentation of microplastics. The fate scenarios were based on literature values and expert estimations. The obtained simplified fate factors were integrated into preliminary CFs and applied to a food container case study. The results confirm the potential relevance of marine litter impacts, the need for developing *physical effects on biota* CFs specific to different types of plastics, as well as the importance of modelling the fate of microplastics in more detail to reduce the uncertainty of CFs.

5.04.T-04 "Superfoods": Measuring the Water-Energy-Food Nexus Under a Life Cycle Assessment Approach

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provide shorter abstract

5.04.T-05 Criteria Used to Review Weighting Methods As Part of the UN Environment Life Cycle Initiative's Global Guidance on Environmental Life Cycle Impact Assessment Indicators (GLAM) Project

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In life cycle assessment (LCA), weighting enables evaluation of the relative significance of impact categories. Notwithstanding the development of several methods over the past three decades, there is yet no consensus as to which methods should be applied and when. This major research question has been taken up as part of the United Nations (UN) Environment Life Cycle Initiative's Global Guidance on Environmental Life Cycle Impact Assessment Indicators (GLAM) project. As part of the GLAM project, a working group has investigated weighting with two main objectives: (i) to develop guidelines for choosing weighting methods for LCA and (ii) to analyse, recommend and apply at least one method (or combination of methods) to obtain at least one set of global weights, which LCA practitioners could use by default when they do not wish to compute or use other weights. As part of the first objective, the subtask identified and reviewed a large set of weighting methods that could be suitable candidates. The methods were grouped into four categories: MCDA (Multiple Criteria Decision Analysis), monetary, data-driven and distance to target methods. Each method was reviewed by at least two team members based on a set of criteria defined by the group, as a step along the way to identifying which methods are most suitable for GLAM recommendations. The criteria are defined as follows: 1. Independence from the set of systems being evaluated, 2. Reproducibility of the weights, 3. Peer review, 4. Method transparency, 5. Coverage of GLAM areas of protection (AoP), 6. Uncertainty characterization, 7. Communicability, 8. Accounting for differences in utility for the same impact, 9. Association with AoP units, 10. Geographical resolution, 11. Global coverage, 12. Application demonstrated in case studies, 13. Required resources to apply the method, 14. Required technical and calculation infrastructure, 15. Representativeness, 16. Bias. Each criterion has been used to assess the different individual weighting methods in terms of their capabilities in principle. An iterative process has been developed in which the criteria have been harmonised, alongside the method reviews themselves. The developed criteria and reviews will be the basis on which the group aims to identify methods that can be recommended and applied to calculate weights at the global level to achieve objective (ii).

5.04 Life Cycle Impact Assessment modeling and application (Part II)

5.04.T-06 Development of Human Health Damage Factors for Climate Change

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Climate change directly impacts human health through complex causal pathways by increasing temperature and precipitation, causing heat waves, fires, droughts, and floods. Indirectly, ecological and social disruptions, such as crop failure or population displacement due to natural catastrophes, are responsible for malnutrition, death by violence, or different mental issues. Those effects are not well covered in existing Life Cycle Impact Assessment methods. Current damage modeling is associated with damage factors covering five main health damage issues, i.e., malaria, diarrhea, cardiovascular diseases, malnutrition, and drowning. Since then, new climate-change-related and epidemiological studies have been published, and there is a need for this knowledge to be integrated into LCIA. Therefore, the aim of this study is to: i) identify all climate change impact pathways leading to human health damages, and ii) provide damage factors for the identified impact pathways by using the most recent epidemiological data. We illustrate this by presenting results of damage factors (DF) due to heat waves. We considered four emission scenarios (RCPs) and two adaptation scenarios in our model (no adaptation and hypothetically full adaptation). Our results show an increase in human health damages due to heatwaves caused by increased radiative forcing. As expected, DF values are lowest when considering adaptation. The lowest DFs are observed for RCP2.6, when no adaptation is considered, due to the lowest temperature increase, and for RCP8.5 when considering full adaptation (thanks to high adaptation measures). Observed variations between scenarios are within one order of magnitude. Compared to LC-impact and IMPACT World+ endpoint LCIA methods, our results show a 20% increase in DFs in case of full adaptation and 519% without, respectively. Our work demonstrates the relevance of better characterising climate change damages, so the LCIA modelling can ensure more accurate results for climate change damage assessment, and hence be better support for decision-making from LCA studies. We thus recommend the implementation of our new human health damage factors for climate change in LCIA.

5.04.T-07 Integrating Dynamic Modelling Into Life Cycle Impact Assessment for Quantification of Biomass Removal by Fisheries

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Fisheries are a globally important nutritional source increasingly suffering from over-exploitation. To improve the sustainability of this non-domesticated global food source, the full extent of impacts caused by fishing activities from the moment of extraction must be well understood. Life Cycle Assessment (LCA) is a method increasingly relied upon to quantify impacts of human activities on the environment and preside over preferred choices for the environments and ecosystems. It is therefore critical that

impact pathways accomplished through Life Cycle Impact Assessment (LCIA) methods are properly quantified. However impact pathways relating to marine ecosystems are a particularly underdeveloped area of LCIA, in comparison to their terrestrial counterparts. Pioneering work has recently proposed a first characterisation of the impact of biomass removal by fisheries on ecosystem quality. A new approach is now presented to develop this characterisation further. Following UNEP-GLAM Phase 3 recommendations to incorporate ecosystem dynamics into the characterisation of this impact, the approach integrates dynamic ecosystem modelling into the LCIA framework. This novel approach enables changes to be captured at the scale of the ecosystem, using an adaptation of time-dynamic trophic model Ecopath with Ecosim. In addition to the direct impacts of biomass removal of target and discarded species, the first output presented from the approach quantifies the subsequent indirect ripple effects through the trophic web, in conjunction with the natural background fluctuations of species. The first results are a midpoint characterisation of biotic change derived from the ecosystem model, allowing the exploration of changes occurring at the ecosystem level. The next step of the approach is its application to Characterisation Factor modelling for the computation of endpoint damage indicators. This can be applied to Areas of Protection (AoP) such as Ecosystem Quality, through a measure of biodiversity loss at regional and global scales, a metric of critical importance within environmental management and conservation decision-making. The outputs are also relevant for application to the Resources and Ecosystem Services AoP. Both pathways provide a step towards highlighting the need for improvement in the balancing act between use and conservation of natural capital and their source ecosystems using LCA.

5.04.T-08 Proposition for a Methodological Framework to Consider Ecosystem Impacts in the Life Cycle Impact Assessment of Offshore Wind Farms

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The development of offshore wind farms is booming with an increasing number of higher and more productive turbines, favored by ambitious roadmaps for energy transition. Life cycle assessment is one of the most complete and mature approaches to estimate the environmental impacts of an offshore wind farm project. Nevertheless, a number of methodological hurdles still have to be overcome to comprehensively analyze the effects of marine renewable energy sector. Among these challenge are the lack of appropriate indicators for the inclusion of ecosystemic impacts during the construction and operation phases of the OWF or the scarcity of ecological data in the marine environment. To address these gaps, we present a methodological framework to model ecosystem modifications due to offshore wind farms and we develop a path to take into account these changes in the life cycle impact assessment (LCIA) step. This framework is based on the identification of impact chains leading to marine ecosystem damage and the identification and characterization of medium-term impacts for which biophysical data exists in the marine environment. This conceptual model, original in its structure, combines both impact chains, classically used in LCIA framework, with an ecosystemic approach, allowing to identify which trophic compartments are affected by which impact chains and the consequences this has on species biomasses. As a result, the characterization of these different impact chains can be translated into new indicators for LCIA using the potentially disappeared fraction of species (PDF) and the potentially modified fraction of functional habitats (PDFH) as measures of the different types of OWF impacts. This framework makes it easier to avoid the risks of double counting in the LCIA framework, to apprehend information gaps and to potentially consider cumulative impacts. It represents then the first step towards the incorporation of OWF impacts on ecosystems in the LCIA framework, that could improve strategic marine planning in the context of marine renewable energy development.

5.04.T-09 Addressing the Triple Planetary Crisis -- a Planetary Boundary Based Life Cycle Assessment of Sugar in Pakistan

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The triple planetary crisis — climate change, biodiversity loss, and pollution — has emerged as an urgent environmental challenge posing an existential threat to human life on planet earth. The production of food is an interesting case in that it is essential for human beings but contributes adversely to these environmental issues. The recent advances in absolute sustainability assessment could help to overcome these issues by identifying opportunities for reducing the environmental impacts within the carrying capacity of planet earth. This study has therefore applied a planetary boundary-based life cycle assessment to sugar in Pakistan. The life cycle impacts were calculated for impact categories including global warming, biodiversity loss, and freshwater and marine eutrophication (i.e., pollution). The study revealed that under the existing production situation, the impact of sugar was within the allowable planetary boundary limits for fresh eutrophication, but not for climate change, marine eutrophication, and biodiversity loss. The enhanced use of byproducts and the use of a more efficient energy system resulted in improving the environmental performance. It could be seen that the use of existing best practices with target driven values could greatly help address the current environmental crisis.

5.04.V-01 Temporally-Explicit Abiotic Depletion Potential (TADP) of Mineral Resources Based on Future Demand Projections

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The depletion potential of metals is one of the main impact categories in life cycle impact assessment (LCIA), and models for assessing it have been heavily debated. Although the depletion potential depends on not only current status but also future status,

such as future demand and recycling, most existing models are based on the only current status. In this study, we extend the abiotic depletion potential (ADP), which is one of the widely used existing characterization models. We propose the extended characterization model: temporally-explicit abiotic depletion potential (TADP), which allows the assessment of depletion potential of metals from different time perspectives considering future metal demand and availability of secondary metals. ADP is one of the main characterization models for mineral resource depletion, quantifying the depletion potential by dividing the extraction rate by the square of a natural stock estimate. In the existing ADP, the current annual extraction is applied for the extraction rate. We extend the ADP by applying the average extraction rate until the target year. The average extraction rates are estimated for 2010-2100 based on dynamic material flow analysis and logistic regression analysis. We calculate the TADPs for six major metals (aluminum, copper, iron, lead, nickel, and zinc) by five SSPs (shared socio-economic pathways), which are represented as relative values against iron (i.e., reference metal is iron). The results show that TADPs for all metals and all SSPs increase compared to those for iron by considering the future changes in total demand and recycling. It implies that the existing models assuming constant extraction amounts (i.e., ADPs) may overestimate the depletion potential of iron compared to the other metals. We show that changes in the relative significance of metals by TADPs compared to the original ADPs may have a great effect on the results of LCIA. Furthermore, we show that the time perspective will affect the relative significance of metals. From the short- and medium-term perspectives, copper exhibits the greatest increase of TADPs compared to the original ADPs (increase in maximum by approximately 1.9 times compared to the existing ADP). On the other hand, from the longer-term perspective, lead and zinc exhibit greater increases in TADPs compared to the original ADPs (increase in maximum by approximately 2.8 times compared to the existing ADP).

5.04.V-02 The Development of LIFE Cycle Impact Assessment Characterization Factors for Desertification

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Along with the acceleration of climate change and expansion from anthropogenic activities, the desertification's impacts are projected to be profound. Drylands area is occupying 42% of earth's surface land with responsible to hold 38% of the global population, which has a significant meaning to our planet. In aims of sustainably developing of society, we applied the LCIA concept to analyze desertification with related impacts so that we can provide a more inclusive quantitative vision for stakeholders. Thus, firstly we proposed the framework for desertification as an extended LCIA midpoint to land use. Then we analyzed and predicted the desertification under different scenarios and try to bridge the characterization factor between inventory to the midpoint. Finally, we incorporated the model from an ecological way to evaluate biodiversity loss as an endpoint to desertification and complete the challenging view of the connection between desertification and biodiversity. As the primary results show the drylands area in 2015 is 53.1 million km². At meanwhile, we compared our results of desertification area with former studies, a report from the united nation for global drylands reported the dryland zone in 2011 was 50.80 million km². As for characterization factors between inventory to the midpoint, the intensity shows a higher potential vulnerability around central Asia to the black sea, northeast Australia and central America, the CF range from -7.62E-07 Ha/tCO₂ to 4.63E-06 Ha/tCO₂ among 5 GCMs. Most of those locations were under a highly warming level and high-water consumption.

5.04 Life Cycle Impact Assessment modeling and application (Poster)

5.04.P-We232 Toward Operationalizing the Fate of Water Consumption in LCA Using Depletion Factors and Tendencies

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Freshwater dependent ecosystems are under multiple pressures, including water resources depletion due to human consumption. Life Cycle Impact Assessment (LCIA) methods usually focus on one aspect of the water cycle (e.g. streamflow), without accounting for its connections to the other compartments of the water cycle. New LCIA methods assessing the impacts of water consumption should reflect the fate of water consumption i.e. the complex transport of water between the water cycle compartments (e.g. surface, groundwater, and atmosphere), so that impacts on different freshwater ecosystems could be modelled. Our study aims to operationalize the fate of water consumption and we illustrate potential applications in LCIA. We developed depletion factors (DF) and depletion tendencies (DT) that reflect long-term consequences of freshwater consumption on freshwater resources available for ecosystems and human communities in four compartments of the water cycle and at the regional scale. DF represent depletion (between 1960 to 2010) of streamflow and groundwater storage and changes in evapotranspiration and soil moisture caused by blue water consumption and are expressed in m³ depletion per m³ consumption in a river basin. DT represent the recent dynamics of depletion (m³.yr⁻²/m³.yr⁻²) showing the change in depletion associated with the change consumption from 2000 to 2010. They are build on the outputs of a state-of-the-art global-scale surface water-groundwater model (PCR-GLOBWB 2.0). We calculated DF and DT for 8,664 basins, covering 93% of the landmass where water consumption is significant (i.e. excluding deserts and permanently frozen areas). Using DF and DT as fate factors would promote harmonization across LCIA methods, and enable new developments providing one element of the characterization factors.

5.04.P-We233 Ecotoxicity in LCA: How to Guide Practitioners Towards Sound Decision Making for Business?

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Impacts on ecosystems from chemical substances emitted to the environment are of growing concern among the population and in business. In LCA, ecotoxicity is often not analysed, and if it is, results are hardly communicated because they are deemed not robust. We aim to answer the question: how to use current ecotoxicity LCA indicators for business decision making? **Objective:** Our objective is fourfold: i) make an inventory of existing ecotoxicity methods and indicators in LCIA; ii) analyse the foundations of these indicators, understand the methodological choices and calculation methods; iii) test methods on case studies; iv) Set up recommendations for practitioners. **Method:** We analyse and compare five methods, using notably GLAM recommendations and criteria as a basis: USEtox 2.12, ReCiPe 2016, LC-Impact, Impact World+ and EF3.0, both in their native format and as available in SimaPro (except for LC-Impact). In order to provide relevant recommendations, i) we map native methodology and CFs' in term of scope (substances covered), impact pathway(s), target media, time horizon(s), underlying data and link with risk assessment methods and data; ii) we analyse the implementation in SimaPro, and related choices made by software developers; iii) we apply methods on substances (3 organics, 2 metallics and 1 inorganic non-metallic) per compartment and sub-compartment and 2 processes (maize grain production and waste water treatment fromecoinvent). **Results:** The lack of terrestrial and marine ecotoxicity indicators in 3 of the 5 methods is changing the way results can be interpreted. For a given substance, native methods and software application do not lead to the same results linked to missing software updates and adaptations of the method, mostly due to absence (or simplification) of spatialization and mapping of emission compartments. We see a notable difference for metals, where time horizon (100 years of infinite) matters. For the two ecoinvent processes studied, we observe differences in results according to the methods used, due to relative importance of CFs between substances and emission compartments between methods. We also compare CFs' underlying data between methods; we notice differences and limitations. **Recommendations:** These results will be followed by recommendations to practitioners, that are still under development. They will cover use of methods and result interpretation, aligned with GLAM and actionable by practitioners.

5.04.P-We234 Problems From Including Technospheric Parameters in Characterization Factors for Natural Resources

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The life cycle inventory (LCI) analysis generally considers a product system in the technosphere, whereas the life cycle impact assessment (LCIA) is generally concerned with impacts in nature. However, in LCIA methods for natural resources, we have noticed a tendency to include technospheric parameters. This practise, which deviates from the predominant use of parameters related to environmental processes in characterization factors for emissions, has not received much attention in the LCA community. Here, we discuss a number of problems arising from such inclusions. Three types of technospheric parameters found in characterization factors for natural resources were analysed: (i) extraction rates, (ii) recycled contents, and (iii) prices. Extraction rates vary over time, and frequent updating is therefore needed to avoid outdated characterization factors. Furthermore, the inclusion of extraction rates in the characterization factors creates an interdependency between the LCI analysis and the LCIA, since extraction rates are also part of the inventory modelling. We show that such interdependencies can potentially lead to counterproductive information. Regarding recycled contents, when inventory data with recycled content are matched with characterization factors also taking recycled content into account, the benefit of recycling is double counted. Furthermore, it introduces a risk of inconsistency: the recycled contents in the characterization factors may not match those in the LCI analysis. In addition, characterization factors based on recycled contents are also time sensitive. Prices are commonly used in economic allocation in the LCI analysis. When they are also used in characterization factors, there is a risk of inconsistency if these prices are not the same as those used in the allocation. In addition, prices are very time sensitive, potentially fluctuating notably even on a daily basis. There are possible solutions to some of these problems, such as frequent updating of characterization factors and avoiding economic allocation. However, these solutions come at a cost. For example, frequent updating of characterisation factors is work intensive, and economic allocation may be otherwise recommendable in some studies. For the LCI-LCIA interdependency, we see no obvious solution. Considering the identified problems, we recommend further critical discussions on the inclusion of technospheric parameters in characterization factors for natural resources.

5.04.P-We235 Impact Evaluation of Circular Economy Strategies Including Resource Dissipation Within Life Cycle Assessment: Literature Review and Methodological Framework

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The increasing level of resource consumption over the past decades led to the development of strategies that fall under the paradigm of the Circular Economy (CE). Life cycle assessment (LCA) is proposed to evaluate the impacts of CE strategies and it is arguably able to support decision-making processes related to the Areas of Protection (AoP) Ecosystem Quality and Human Health; however the same level of consensus is not applicable when measuring impacts in the AoP Natural Resources. Various methods address the impacts of mineral resource extraction, focusing on the questions of resource depletion and criticality; however, Resource Dissipation (RD) remains under discussion; there is a limited number of proposals to assess the impacts of dissipative losses. As part of the DissipaLoop project, supported by the French Alternative Energies and Atomic Energy Commission (CEA) and the French Geological Survey (BRGM), this contribution aims to identify methodological gaps and provide recommendations on the evaluation of CE strategies through LCA to support decision-making processes; for this case, the introduction of a recycling technology. Three literature reviews were conducted. One on the links between LCA, CE and RD. Next, an extension of the existing reviews on CE followed by a multi-criteria process of classification to select metrics that support the objective of the study. Finally, a review on RD was extended to identify developments on the impact assessment of dissipative flows. A methodological framework was constructed for the evaluation of CE strategies through LCA. The study of CE

metrics allowed selecting seven that could inform on the impacts of these strategies. Among the assessment methods focused on RD, it was identified that an unexplored pathway is the measure of lost value due to potential resource dissipation. The development of the framework allowed identifying how the concepts of CE, LCA and RD connect, with special focus on the methodological gaps for the evaluation of CE strategies. From the wide range of studied CE metrics, seven can be suggested in connection with LCA to inform the decision-making process regarding new recycling technologies. Finally, an impact pathway focused on potential lost value due to RD was identified. Future work will focus on the development of the identified impact pathway in RD and its testing within the evaluation of a novel recycling technology through LCA and the application of the suggested CE metrics.

5.04.P-We236 Split Gas Approach to Set New Emission Reduction Targets Compatible With 1.5 °C Warming in the Agri-Food SECTOR

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5.04.P-We238 Water Footprint of FORest and Orchard Trees: Methodological Gaps and Trends

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The current challenge of global water scarcity is addressed in Goal 6 of the 2030 Agenda for Sustainable Development ('Ensure availability and sustainable management of water and sanitation for all') aiming to increase global water-use efficiency and ensure sustainable water withdrawals and supply. According to the current water use planetary boundary, which focuses on allowable blue water consumptive use, water use is categorized as a global safe operating space, in spite of boundary transgression risk in some regions. However, it has been highlighted the need of understanding how climate and living ecosystems respond to changes in the different forms of water on Earth. Trees have a relevant role in the water cycle. They can be rain-feed, use groundwater water and, at small scale, be irrigated. Blue water includes surface and ground water, while green water represents rain water. Trees also release water through evapotranspiration (ET) and, thus, disturbances in tree management affect ET and, consequently, atmospheric moisture transport and water availability. The aim of this review is to identify how water footprint (WF) applied to trees (forest and orchard trees) is currently being addressed, and which are the methods used, methodological gaps and main challenges for a consistent and harmonized WF scarcity assessment. The review used different databases as search engine, focused on studies published in peer-reviewed journals, considering keywords focused on forest and orchard trees, WF measurements and methodological choices. The final articles sample (47 publications) was categorized as case study (35), methodological development (1), overview (4), mixed approach (5) and review (1). Trees located in Europa (with Italy in the lead) and Asia represented the highest number of studies, followed by global scale analysis. Olive and oil palm trees were the most analyzed within case studies and mixed approaches, while forest was covered in overviews, methodological development and review. Spatial and temporal resolution were features indicated in almost 40% and 90% of the articles sample, respectively. This review shows that for obtaining a consensual and harmonized assessment of the WF scarcity of forest and orchard trees there is still a pathway to be followed. Further methodological improvements are crucial for a better understanding of the relationships between green and blue water in agroforestry systems, including the effects of edaphoclimatic conditions.

5.04.P-We239 Introducing Impact of Marine Plastic Debris on Ecosystem Service to LCIA

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Around 40% of the world's population nowadays is located in the coastal area, whose life is closely associated with marine ecosystems and their services, such as food provision, storm protection, recreational opportunities and biodiversity. However, marine ecosystems are increasingly confronted with many anthropogenic stressors such as plastic debris and invasive species, affecting many ecosystem services. Unfortunately, an operational impact category for marine ecosystem services within the framework of life cycle impact assessment (LCIA), is missing, leaving a knowledge gap between current status and ecosystem protection policy. We try to narrow this gap, by building an effect factor for microalgae's carbon sequestration in response to marine plastic debris, as marine microalgae are responsible for 40% of the total CO₂ absorption in the biosphere. In our study, we collect growth inhibition rate data for different microalgae and polymers with different sizes and concentrations. This data is used to construct spatial-differentiated species sensitivity distributions (SSDs), which are used as the basis to calculate effect factors of carbon sequestration loss. The result provides the first effect factor quantifying impact on ecosystem services. In addition, our results lay the foundation for future marine ecosystem services assessment in LCIA. The next stage of our work will be to develop a fate factor, which will enable the development of complete characterization factor for impacts on carbon sequestration.

5.04.P-We240 Hybrid Consumption-Based Normalization Approach for the Life Cycle Interpretation in the Plastic SECTOR: A Proposal

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Life cycle assessment (LCA) is widely used for the comparative assessment of the environmental performances of materials and processes. The strength of LCA is the ability to analyze impacts in multiple environmental aspects throughout the life cycle of the system being analyzed. If the objective of the study is the analysis of the environmental profiles of a material as well as the decision support in the comparison and choice between different alternatives, tools for achieving a synthesis of multicriteria results become necessary. In this regard, the LCA reference standards describe the optional normalization (calculating the

magnitude of the indicators of categories relative to the reference information) and weighting procedures. Even if practitioners perceive normalization and weighting process negatively due to uncertainty and loss of robustness, their contribution to the interpretation and decision making of results is recognized. Typical problems of external normalization are related to uncertainty linked to the data of the reference system, discrepancies between the substances of the two life cycle inventories (analyzed system and reference system), inverse proportionality and the large amount of data required. The aims of this work are: (i) present a hybrid (internal-external) consumption-based normalization approach applicable in the context of cradle-to-gate analysis of plastic materials based on the annual European plastic demand, (ii) evaluate the variability of the normalization set considering the years 2005 and 2019 as reference. The weighting procedure is not addressed in this work. For this work, aiming to present normalized environmental profiles (cradle-to-gate, declared unit 1 kg) of three plastic materials (PP, PVC, PLA) a normalization set was built on the basis of the European plastic demand starting from the data provided by Plastic Europe for the year 2005 and 2019 and the secondary data proposed by Ecoinvent 3.6. Sets of impact categories and methods proposed by PEF were used. The presented normalization approach is a proposal for a better interpretation of LCA results in the plastic sector. Due to the limited amount of data required, continuous updating over time is possible, as well as the establishment of a reference in line with the goal and scope of the study. The use of common secondary data and methods allows us to address the main problems associated with external standardization approaches.

5.04.P-We241 Integration of New Climate Change Characterization Factors for the Aircraft Life Cycle Assessment

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Air traffic accounts for 11% of world fuel consumption and 2% of world CO₂ emissions (IATA, 2017). The cumulative emissions from 1940 to 2018 are 32.6 billion tons of CO₂ (approximately 50% have been emitted in the past 20 years) (Lee et al., 2021). Most of these emissions occur during the cruise phase. Life Cycle Assessment (LCA), is used as a reference method for air transport environmental assessment. As a regulatory reference, Global Warming Potential (GWP) is nowadays the most strategic impact category for air transport. Aircraft emissions are mostly located at the level of the upper troposphere and the lower stratosphere during the cruise phase according to the flight of the aircraft (courts, medium and long haul) (IPCC, 1999). Emitted molecules have a different radiative forcing and as a consequence a different impact on climate change depending on the altitude at which the molecule is emitted (Wahner et al., 1995 ; IPCC, 1999 ; Jungbluth and Meili, 2019 ; Matthes et al., 2021). This specification is not currently considered by the LCA characterization method (IPCC, 2013) which only considers that emissions are on the ground. The objective of this study is to integrate the impact characterization factors specific to high altitude emissions into the LCA. The functional unit consists of transporting a person over a distance of one km in a long-haul aircraft using conventional fuel (jet type A-1). The plane, the airport, the kerosene, the ground emissions, and the high-altitude emissions are considered. The characterization factors as well as the high-altitude emissions from Lee et al. (2021) are integrated. The results show that considering Lee et al. (2021) high-altitude emissions and characterization factors increase GWP impacts by 102%, from 9.79 E-02 kg CO₂eq (kg of CO₂ equivalent per person per km) for the base case scenario compared 1.98E-01 kg CO₂eq/pkm with Lee et al. (2021) values. High-altitude emissions are strategic for LCA of air transport as it represents the biggest part of the total impact. For Lee et al. (2021) scenario, high-altitude emissions' impact represents 91% of the total impact (plane, airport, kerosene, ground and high-altitude emissions). It represents 82% for the base case scenario. This increase is mostly due to the integration of Lee et al. (2021) new characterization factors of nitrogen oxides (NO_x) which had not been calculated for the emissions at altitude and which is now at a GWP of 114.

5.04.P-We242 Towards a Safe Circular Economy - Monitoring Strategy for Substance of Concern

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The EU's chemicals strategy for sustainability aims for a toxic-free environment. In addition, the current linear economy should transition to a circular economy to deal with material use and associated environmental impacts. In such an economy, raw materials are continually reused in order to minimise waste. Examples of developments in the transition to a circular economy include the increasing use of separate collection and recycling, but also the emergence of new chemical recycling methods and the renewed focus on re-using and repairing products. However, (reused) materials can contain hazardous substances, which can harm the environment and human health. As long as substances of concern are used in products and materials, these substances could re-enter the product chain in a circular economy. It is crucial for a safe circular economy to know what will happen to substances of concern when products, parts and materials are used in a subsequent application. This is particularly relevant if the new application is different from the original use. This might lead to new unintended exposure of workers, users and the environment to substances of concern. Three major challenges for a safe circular economy are identified [1]:

- Availability of information on substances of concern in the supply chain
- Expanding responsibility throughout the entire product chain
- Safe handling of substances of concern in a circular economy where phasing out is not possible

All in all, a circular economy is not necessarily safe. However, the transition to a circular economy requires a different approach to substances of concern, and offers momentum to take a safer, more preventive approach to coping with these substances and their risks. It is important to know whether a product or material is safe for people and the environment. However, it is not easy to get a complete overview of substances of concern in materials. There are many kinds of these substances that are present in a many different materials and products. RIVM has prepared a draft method for determining and monitoring the risks posed by substances of concern in a circular economy [2]. This monitoring method makes it possible to analyse at which location in the value chain of production, use, and (waste) processing substances of concern can be present and where they may cause risks. Using these insights, it is for example possible to show how government and industry can make efforts for safe processing of materials.

5.05 Prospective assessment of emerging technologies and emerging product systems in a life cycle perspective (Part I)

5.05.T-01 Prospective LCA of Bio-Based Products: A Scenario Methodology and Lessons Learnt From the PLANET BIO Project

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Prospective LCA aims at evaluating the potential impacts of future technologies and thereby guiding their development. Within the project “Prospective LCA for Novel and Emerging Technologies for BIO-based products” (PLANET BIO), state-of-the-art methodological guidance was compiled to perform prospective LCA for bio-based products. More specifically, guidance addresses aspects that are particular to prospective LCA across the 4 phases of LCA, the development of scenarios, the modeling of biogenic carbon flows, and the handling of uncertainties. Here we provide an overview of this guidance. We then focus on one aspect particularly, which is a novel methodology for the development of scenarios, which often lacks an explicit treatment in prospective LCAs. We present a practical framework for developing scenarios and conducting scenario analyses that should help LCA practitioners to a) develop better scenarios and b) be more explicit in describing how the scenarios were developed. This should not only increase transparency and reproducibility, but should also help the reader to better understand which parameters may be key in different possible futures and to foster the discussion about possible future developments rather than just focusing on LCA results relating to these. The core scenario methodology consists of 4 steps as part of the inventory analysis phase: i) the identification of key parameters, ii) the development of specific sub-scenarios for each parameter, iii) the creation of overall scenarios and narratives, and iv) the implementation of these scenarios in LCI models. Specific tools to support the work in each of these steps are described (e.g. cross-consistency analysis and concepts borrowed from morphological analysis). A key element of the work is the combination of a causal loop diagram with an LCA flow chart, which aims at linking in a visually explicit way the technology parameters, big-picture surrounding parameters, and the LCI model itself. This diagram can be a key element during scenario development, but also for the further communication of scenarios to different stakeholders.

5.05.T-02 Methods to Environmentally Assess Cleanroom Facilities for Emerging Technologies

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Predictive LCA is a valuable tool for anticipating and ideally avoiding the environmental impacts of new technologies. Many of these technologies, especially those involving nanoscale processes such as semiconductor manufacturing, require defined clean spaces to ensure high quality end products. However, the facilities that function as cleanrooms are not well standardized, only the output that a cleanroom provides must meet a set of defined standards. The lack of standardization in cleanroom design presents a challenge for LCA analysts, who have only a handful of outdated sources, limited LCI data, and few studies with real measured data to draw upon. Such challenges do not allow the LCA analyst to effectively quantify, prevent, and minimize the environmental impacts of new technologies that require the use of cleanrooms. Since cleanroom maintenance involves a significant amount of energy, the lifecycle steps that must take place in these defined spaces can be an important factor in evaluating the overall sustainability of incumbent and new technologies. By considering parameters such as energy requirements for cooling and heating, or future energy mixes, we explore a large solution space of possible combinations. All of these different scenarios allow us to quantify the impact of cleanrooms under a variety of real-world conditions. We were able to identify possible trade-offs between relevant parameters depending on ISO classification, location, and climate. For example, environmental impacts are closely related to the locally available energy mix. However, we can show that for ISO classes 6 to 9, energy requirements for heating and cooling can play an important role in the choice of cleanroom location. Depending on the required performance, we can directly rule out scenarios with very high and undesirable environmental impacts. In addition, we provide usable LCI data and scenarios that can support the LCA analyst in need of such data. Beyond this specific case study, we also provide a combination of techniques that can streamline the evaluation of systems that depend on a variety of parameters. Our work also aims to broaden the focus of cleanroom designers on the sustainability of this service and whether, for example, location plays a role in deciding where to establish and operate future cleanrooms.

5.05.T-03 Dealing With Future Developments of Wind Offshore Energy for Integration Into an Energy System: How to Incorporate Environmental Aspects?

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Life cycle assessment (LCA) is a standard methodology to assess the environmental impacts of technologies. Many LCA studies prove that wind energy has a low impact on climate change and, thus, is often suggested for the decarbonization of the electricity sectors in different countries. In addition, the LCA studies provide indicators of the environmental performance during the construction, installation, and operation of wind turbines. Yet, these indicators might capture technology development of wind energy meagerly because of the LCAs static approach that depends on the database for background data which is often much older than foreground data. In the meantime, technology evolves and innovates. That is why the integration of LCA indicators into energy systems models is not straightforward. Energy system models provide scenarios that address different visions of the future. Therefore, the environmental aspects of energy systems should grasp technological development, if possible, from two perspectives. First, the life cycle inventory (LCI) should include changes in the foreground according to the future development of the most relevant parameters. Second, the LCI

should consider technical and temporal adjustment of background processes, like the market share of resources and utilities. This study focuses on the inventory of wind offshore electricity generation and considers upscaling of wind turbine components. This work applies the scenario development method for wind offshore energy in Germany using the Superstructure approach in the LCA opensource software Activity-Browser, which employs future LCI provided by the Premise initiative. The study sets up scenarios adapted for Germany to reflect expected future trends for wind offshore. The aim is to obtain specific impact indicators and integrate them into an electric system model for assessing optimal operation strategies for future power plant fleets regarding economic, technical, and environmental aspects.

5.05.T-04 Application and Adaptation of a Scale-Up Framework for Life Cycle Assessment to Resource Recovery From Waste Systems

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Introducing Life Cycle Assessment (LCA) in the design stage enables determining hotspots in an emerging technology and may lead to process improvements. Full-scale plant data is not yet available for emerging technologies at low technology readiness levels, which is one of the major challenges of early stage LCAs. This study tested an engineering-based upscaling method, adapted it to biochemical resource recovery processes, and checked the validity of the results as compared to data from real full scale operations. The study displays substantial difference between the methane production amount in actual plants (scenario 1) and conceptual plants based on raw lab-scale data (scenario 2). On the other hand, when a conceptual plant was designed based on pilot plant data or modified lab-scale data taking into account the reaction kinetics (scenarios 3-4), the LCIA results of the conceptual cases were reasonably similar to the actual case. For those scenarios, it may be claimed that the difference in the results are fairly limited and within ranges that can be expected. In line with other studies [6] it is recommended to be careful when comparing emerging technologies with their incumbent counterparts not to unfairly disqualify emerging technologies in their early design stages.

5.05 Prospective assessment of emerging technologies and emerging product systems in a life cycle perspective (Part II)

5.05.T-06 Technology Learning Effects in Prospective LCA: Rule of Thumbs Derived Using LIFE Cycle Inventory Databases

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Considering environmental impacts in the early stage of product design gets increasingly essential. This is because degrees of freedom in the early design stage are greater than in a later stage of design. On the other hand, uncertainty decreases throughout technology development as decisions are taken, and the observed process becomes clearer and more data is available. In order to provide data in an early design stage and scale this data to a future point in time, upscaling schemes are needed to fill data gaps. A method for upscaling are so-called learning curves. Learning curves can examine historic development rates of technologies and can be extrapolated into the future to estimate technology learning effects. This approach has already been used in economics to analyze the correlation between production and costs and preliminary to the development of environmental impacts. In this work, different versions of the LCI database ecoinvent are used to derive scaling factors. Each of these databases is analyzed using the Brightway 2.5 LCA framework by calculating the environmental impacts of each process. Via mapping processes producing the same reference product in the same geographical location of each database, the environmental impacts of each process can be compared throughout the databases. Following this approach, it can be concluded that, for example, the GWI is by 0.5 % when comparing ecoinvent 3.1 with ecoinvent 3.8. The results show the applicability of the method and indicate potential technology learning over time based on LCI databases. These findings will be helpful in the application of prospective LCA. However, product- or sector-specific scaling factors could lead to an even more insightful analysis.

5.05.T-07 Modelling Biological and Techno-Operational Uncertainty in Ex Ante LCA of Microalgal Molecule Productions

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On the ground of their mostly unexplored phylogenetic and biochemical diversity, microalgae are being explored in a search for new high value and promising molecules such as food supplements, nutraceuticals or pharmaceuticals. The literature shows a high variability of the environmental impacts associated to microalgae products due to diversity of strains, locations, cultivation periods and technologies. As no industrial scale process data is available for microalgae, LCA studies rely either on laboratory and pilot scale results or on assumptions on productivities in given production systems. However, we do not know to what extent the assumptions and results from these previous studies can be generalized to novel strains. Biophysical models have shown how different strains behave and grow distinctly in different photobioreactors (PBR). For a novel strain and molecule, we thus cannot forecast yet which PBR geometry and which operational parameters (biomass concentration and flow rate) will allow a certain productivity. To integrate this uncertainty in the Ex-ante LCA of novel microalgae products, we propose and test a parametrized and consequential LCA model combined with a stochastic approach. The deterministic model relies on 60 interacting and independent parameters covering the biological, techno-operational, physical and geographical dimensions and dynamically simulates the microalgal molecule production in any location and season. An extended stochastic Fourier Amplitude Sensitivity Test (FAST) is performed to propagate the uncertainty on 6 parameters associated to the PBR design, operation and

thermoregulation to the environmental impacts. The model is tested for a realistic strain cultivated in Aalborg; Denmark and Granada; Spain and shows that thermoregulation is a key and overlooked environmental hotspot, both in absolute value and in uncertainty. The results highlight the necessity, for Ex-ante assessments, to go beyond simplistic assumptions on areal productivities as little variation of the cultivation system and operation may lead to significantly different impacts. Despite uncertainty, the model shows little overlapping for the environmental impacts between the two locations and thus demonstrates its applicability as a decision-making tool. This work and model eventually aim at exploring the future environmental impacts of microalgae productions through comprehensiveness, uncertainty integration and transparency.

5.05.T-08 LCA of Emerging Transportation Systems in Pilot Operation Including Scenario Analysis

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Mobility solutions with low climate impact are needed to reach a sustainable transport sector. In order to decarbonise the sector, a large number of emerging e-mobility technologies are currently being developed, in which the energy source is changed from fossil fuels to electricity. Life Cycle Assessment (LCA) is used as a tool to evaluate these technologies regarding their contribution to achieve the climate targets in the future. However, different technologies have different technology readiness levels (TRL) and moreover, the underlying mobility concepts will evolve during the process of technology development and early phases of application. Consequently, this has to be included in the ecological assessment for a comparison to established transport technologies. Our work shows a comparative LCA of the two freight transport alternatives *conventional truck* and *overhead line hybrid truck* (OH truck). The data input comes from the implementation and operation of a real field trial in Germany. The field trial, where local transporters deliver goods from the depot to the destination over the realised eHighway track of 5 km in each direction, provides pilot operation data. From this data, an inventory is created and a reference LCA is performed, which refers to TRL 6 of the field trial. In a second step, the approach is extended to a possible development state that includes real-world application of TRL 7. To perform this, a scenario approach is developed. In the scenario approach, the deployed eHighway track remains the same, but with higher vehicle utilisation. For the OH truck, several technological improvements are assumed. These future improvements consider the transformation potential of the technology under development. Despite the varying data availability and uncertainty associated with emerging technologies, it was possible to identify and model the future performance of the new transport system. The results obtained from a transparent and structured methodological approach could increase the comparability of the two transport alternatives. The outcomes show large differences between the actual state assessment and the assessment taking into account the scenario approach. By considering future improvements of the emerging product system, greenhouse gas reductions amounting to half of the budgeted climate targets can be determined for the OH truck compared to conventional freight transport.

5.05.T-09 Parameterized Life Cycle Inventories for Representative Life Cycle Assessments of Future Energy Scenarios: Application to the French Heating SECTOR

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Life Cycle Assessment (LCA) is a worldwide accepted methodology to estimate the environmental impacts linked to the life cycle of a product or service. Despite its standardization, practitioners face challenges when applying LCA, especially in the field of future technologies. A major one relates to integrating future systems within an ever-evolving background, e.g. changed composition of the supplied electricity to future technologies. Coupling outcomes of Integrated Assessment Models to foreground LCA systems is one solution sometimes applied. While being extremely comprehensive, this computation-intensive approach does not offer the flexibility to easily assess the influence of changes in background elements on the environmental impacts of the studied foreground system. Here, we show how life cycle inventories of background processes can be parameterized and combined to parameterized foreground inventories to estimate the environmental impacts of future energy production and consumption trajectories. The evolution of the French heating sector until 2050 is used as case study. First, parameterized LCA models of electricity and gas production technologies likely to play a role in future energy mix were developed based on 7 to 28 parameters each for a functional unit of 1kWh of energy produced. Second, parameterized LCA models for heat pumps, gas and oil boilers, electrical heater, district heat, and wood heater, thus the foreground, were defined using 1 to 6 parameters each. The modelling relied on the *lca_algebraic* library, a new layer above the *Brighway2* open source Python library for LCA. Third, the background and foreground LCA models were combined. Finally, the environmental impacts of three consumption trajectories of the French heating sector coupled with two power mix scenarios were estimated using the combined parameterized LCA models. The consumption trajectory relying on an increased energy sufficiency trajectory had less impacts on freshwater eutrophication, ionising radiation, acidification, and climate change than the other two trajectories representing an evolution following the technological developments of the French Environmental Regulation 2020 and a lower efficiency respectively. This was observed for both power mix scenarios. The parameterization and combination of background and foreground LCA models allows to flexibly estimate the environmental impacts of future technologies or systems while ensuring their representativeness.

5.05.T-10 Prospective Life Cycle Assessment of a Cobalt Fischer-Tropsch Catalyst

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The Fischer-Tropsch (FT) catalyst production, use, and end-of-life phases are often omitted or simplified in Life Cycle Assessments (LCA) on FT products since the data required to model these stages are generally not publicly available.

Consequently, the effects of the catalyst on the whole life cycle of FT liquids are not known. This study presents the prospective LCA of a novel Co-based FT catalyst, in the scope of the REDIFUEL project (funded by European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 817612). The main objectives were to evaluate different future production pathways and identify a best-case and a worst-case scenario. The foreground was modelled with upscaled data from lab-scale experiments and patents. Different hydrogen production sources were considered, and the effects of two different end-of-life scenarios (regeneration and recycling) were analyzed. The assessment considered the years 2030 and 2050 and a superstructure prospective background database was constructed with the Python library premise. The selected storylines for this database were the SSP2-base (no climate policy) and SSP2-pkbudg900 (1.5 °C climate target). The results showed that the climate change impact of the catalyst depends mainly on the end-of-life scenario chosen. Regeneration and direct reuse of the catalyst led to a 65-80% lower impact than recycling. Using different background scenarios lowered the impact by 10% (SSP2-Base-2030) to 38% (SSPS2-PkBudg900-2050) compared to the Ecoinvent database. The hydrogen source affected the results too. Of all options, H₂ from autothermal reforming of natural gas had the highest impact. The best case for Co-based FT catalyst production is the scenario with regeneration at the end-of-life and ATR of biomass for hydrogen production consumed in all processes. In the worst-case, the catalyst is recycled with metal recovery, while the hydrogen is produced via autothermal reforming of natural gas. Although the prospective background database had an important effect on the results, the different end-of-life options were more influential, making this life cycle stage the primary concern of a prospective LCA of Co-based catalysts. The next steps in this work are analyzing other environmental impact categories and assessing the contribution of the FT catalyst to the bioproducts life cycle.

5.05 Prospective assessment of emerging technologies and emerging product systems in a life cycle perspective (Virtual Only)

5.05.V-01 Goal and Scope Definition of Life Cycle Assessment Applied to Emerging Technologies: FineFuture Flotation in Mining Industry

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Froth flotation is one of the most frequent methods employed to concentrate precious minerals for downstream refining stages. Currently an enhanced flotation technology is being developed under FineFuture (FF) project, funded by the European Union Horizon 2020 research and innovation programme (grant agreement No 821265). The project will advance the basic understanding of fine particle flotation phenomena (below 20 µm in size) and lead to the creation of ground-breaking technological solutions. If proves effectiveness, this technology will have the potential to valorise fine mineral particles rather than disposing them as waste. However, this does not necessarily guarantee the technology's environmental sustainability. While Life Cycle Assessment (LCA) is a consolidated method to assess potential environmental impacts of in-operation systems, current LCA framework exhibits some challenges when applied to emerging technologies. In this proposal, we show the decision-making process in the goal and scope phase, and data collection approach applied to two industrial case studies within FF project. The first case study is Grecian Magnesite whose main products are magnesite concentrates (MgCO₃) and magnesia (MgO). The innovative flotation technology is expected to be used on residual fines (< 4 mm granular size) from beneficiation washing units that are now unexploited, or on low-quality MgCO₃ concentrates (< 90% MgCO₃) after beneficiation stage to acquire higher concentrations of MgCO₃. The second case study is Eramet for manganese (Mn) concentrate production. The technology here will target tailings which are the discarded residues of manganese beneficiation process, yet they contain a considerable amount of Mn. For both case studies, the LCA is a comparative one between current systems of beneficiation, and future system with FF flotation implemented. By applying LCA in this way, it will be possible to assess the most environmentally sustainable solution and identify any shortcomings of the emerging technology in early technology readiness level (TRL). LCA can be a reliable evaluation tool for novel technologies if methodological challenges could be overcome. Real-world studies like this can push towards methodological developments of LCA based on real experience of current method's drawbacks. These developments can provide a solid foundation for furthering the application of LCA to emerging technologies by confirming its effectiveness in real-world cases.

5.05.V-02 Life Cycle Assessment of a Heterogenised MOLECULAR Complex on Porous Carbon Support With Poly-Ionic Liquids Incorporation for CO₂ Electroreduction in Water/Ionic Liquid Media

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Electroreduction of CO₂ is one of the ways to valorise CO₂ as a source of carbon. The development of molecular catalysts for CO₂ electroreduction within electrolyzers requests their immobilisation on the electrodes. While various methods have been explored for the heterogenisation of homogeneous complexes, mainly introducing functionalities to the molecular catalysts, we here report different porous bio-inspired carbon materials as a support for a well-known molecular catalyst with the particularity in addition to exploring its environmental profile. This cathodic hybrid material has been tested for CO₂ electroreduction using a mixture of an ionic liquid and water as the electrolyte. The feasibility of such a system on an industrial scale needs a study focused on a low environmental and economic impact process. A life cycle assessment (LCA) analysis has been performed considering the three different carbon materials scenarios and evaluating the more environmentally friendly to incorporate the environmental perspective to the product development. A cradle to gate approach was used, trying at the same time to overcome the limitation about the low technological readiness level (TRL) of the developments. The experimental best-case scenario has been further studied to see the hot spot process/material for the LCA analysis of the CO₂ electroreduction. Since the cathodic hybrid electrode

catalyses the conversion of CO₂ into a mixture of carbon monoxide and formic acid, with a selectivity that depends on the applied potential and the water content in the electrolyte, the different CO₂ electrocatalytic scenarios are studied. The best scenario is identified by considering the variation of the most important parameters such as potential applied, current density obtained, products selectivity and water content in the electrolyte. These results emphasise the advantages of integrating molecular catalysts onto such porous carbon materials for developing novel, stable and efficient, catalysts for CO₂ reduction, which allow the low loading of noble metal as a catalyst and pave the way toward a more scalable process.

5.05.V-03 Life Cycle Assessment of Bioinspired Electrode Materials for Hydrogen Evolution, Alternate to Carbon-Based Economy

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Water electrolysis using solar energy is regarded as one of the most sustainable techniques for hydrogen production due to its relatively low emissions of greenhouse gases^[1]. However, current electrolyser technologies for the splitting of water into O₂ and H₂ usually rely on the use of rare and expensive platinum at the cathode for the hydrogen evolution reaction (HER) and iridium or ruthenium at the anode for the oxygen evolution reaction (OER)^[2]. In nature, [FeFe] hydrogenase enzymes have attracted tremendous attention for their reversible hydrogen production with remarkable catalytic rate (6000–9000 molecules H₂. S⁻¹ per site) using only earth abundant metals^[3]. Industrial usage of such enzymes is limited due to their extreme oxygen sensitivity and fragility^[4]. However, they became valuable blueprints to develop bioinspired catalyst for large-scale hydrogen production as a promising alternate to carbon-based economy^[5]. In order to make a durable and efficient solar-fuel conversion and integrate such catalysts into an industrial scale, it is necessary to combine bioinspired system with functional support (electrodes, semiconductor, nanoparticle, etc.)^[6]. However, despite the importance of assessing the environmental impacts^[7] of such system, studies based on sustainability of such bioinspired system are yet to be reported. Our recent work focuses on the preparation of oxygen stable hydrogen evolution catalyst (HEC) by embedding [FeS(CO)₃]₂ core, a functional mimic of native enzyme, within functional polymers structures, based on recently reported metallopolymers^[8]. Specifically, we are developing a methodology to anchor these metallopolymers onto nanostructured electrode substrates such as multiwalled carbon nanotubes (MWCNTs) in a stable manner. Our final goal includes exploring the sustainability of resulted system (electrode-catalyst-electrolyte) through Life cycle assessment (LCA). This has allowed thorough ‘Simparo’ software considering the system boundaries of ‘cradle to gate’ and functional unit of ‘1 kg of H₂ production’ using ‘Recipe 2016 midpoint’ as a calculation method. Our preliminary results revealed that the newly synthesised metallopolymer on MWCNTs showed high improvement with two times higher turnover number (TON), more than twice durability for hydrogen production under catalytic condition and three times lesser environment impact for ‘1 kg of H₂ production’ as compared to that of previously reported metallopolymer^[8]. **References** 1. Appl. Energy, 2019, **237**, 862–872. 2. J. Am. Chem. Soc., 2015, **137**, 4347–4357. 3. ACS Catal., 2020, **10**, 7069–7086. 4. Proc. Natl. Acad. Sci., 2009, **106**, 17331–17336. 5. Sustain. Energy Fuels, 2018, **2**, 724–750. 6. Nat. Nanotechnol., 2018, **13**, 890–899. 7. Green Chem., 2015, **17**, 123–145. 8. ACS Macro Lett., 2018, **7**, 1383–1387.

5.05.V-04 Measuring the Reuse Potential of Building Materials: Literature Review and Development of a Reuse-Potential Indicator

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The Building and Public Works sector is responsible for half of the extraction of raw materials and energy consumption at European scale. It is also responsible for the production of 227 million tons of waste in 2014 (43 Mt for buildings in France including 23 Mt resulting from demolition). In order to remediate the resource-intensive consumption of this sector (and all sectors in general), waste hierarchy has been introduced by the European Union since 2008. It prioritizes reuse of waste over recycling. Hence, reuse is considered as the best waste recovery method at the end of the life cycle of an object and it is promoted by governments. In this context, many initiatives have emerged in the field of building materials to attempt to generalize reuse practices (Interreg FCRBE, Rotor, Mobius réemploi...). One of the challenges of this issue is to identify and measure the reuse potential of building elements. The BAMB project (Buildings As Material Banks) defines reuse potential as a measure that expresses the likelihood that the parts of that assembly can be disassembled simply, fast and without damage, and thus reused. In this work, a quick literature review on reuse potential will be presented, with its definition(s) and initiatives to construct an indicator of reuse potential of objects. Mains strengths and weaknesses of such indicators will be highlighted. Finally, based on observations from the literature review, we will develop a reuse-potential indicator adapted to load-bearing building components. Thereby, the study will highlight advances in current research and add a contribution on reuse-potential indicators for building materials which are a key element to spread and generalize reuse practices.

5.05.V-05 Proposition of a Life Cycle Assessment Study for the Synthesis of Proton-Conducting Membranes for the eSCALED Polymer Electrolyte Membrane (PEM) WATER Electrolysis Device (Artificial Leaf)

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The proton-conducting membrane is a key component of PEM electrolysers. Indeed, the membrane prevents electron transport and mixing of components coming from both electrodes. The thinness of the membranes allows a better transfer of protons between both electrodes. To be effective, the membrane must be durable. Meaning that it should have a good chemical, and mechanical stability; a high proton conductivity and be compatible with the electrode's materials. To date, the commercially available PEM displaying good performances is Nafion®. However, Nafion® fabrication is a complex multistep process. This

factor contributes to the high cost of the membranes. In the matter of reducing the cost of PEM while improving the conductivity, mechanical and thermal properties of the membranes, we developed a membrane based on partially sulfonated poly(pentafluorostyrene) (PPFS) and a second polymer providing more flexibility to the membrane. Membranes were made with poly(butyl acrylate) (PBUA) and bio-based poly(menthol acrylate) (PMA) as second materials. Block and statistical copolymers were synthesized using both copolymer systems. With lower amounts of sulfonated PPFS and thanks to materials' organization, block-copolymers were found to have better conductive properties than their statistical counterparts. Life Cycle Assessment (LCA) methodology has been applied to evaluate the environmental impact and accumulated energy requirements related to the manufacturing of the eSCALED proton-conducting membranes. This assessment allows investigating and identifying the environmental impact hotspots of membrane manufacturing process, thus, enhancing the sustainability of the technology in its future upscaling. The cradle-to-gate LCA being performed compares the environmental impacts of two different synthesis techniques to obtain sulfonated block and statistical PPFS/PBUA. Moreover, by using less PPFS in the case of block copolymers, the overall environmental impact of the membrane should be lowered. Additionally, our goal is to determine if using a bio-sourced polymer (PMA) better the environmental impacts of the membrane elaboration compared to PBUA. Our preliminary results showed that at a laboratory scale, the highest impact comes from the energy inputs used for the synthesis of polymers and not the actual chemical used to make the membranes. Hence, reducing energies used during polymerization could help optimize the membrane elaboration processes.

5.05 Prospective assessment of emerging technologies and emerging product systems in a life cycle perspective (Poster)

5.05.P-Th156 Prospective LCA of Perovskite Solar Cells for Building Integrated Applications

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Perovskite solar cells (PSCs) are innovative photovoltaic devices that are expected to become commercial in the near future. For this reason, scientific research is doing a great effort to improve their lifespan, power conversion efficiency (PCE) and stability. On the other hand, industrial research is trying to optimize the fabrication of these devices by reducing the energy and the materials demand for their manufacturing; moreover, the analysis of PSCs real world operation is another important research topic. The study presented in this abstract is a prospective life cycle assessment (LCA) where future scenarios related to the technological development of PSCs and to the industrial scale-up of their manufacturing are used to predict the potential environmental benefits of this technology. Particularly, we assume that PSCs are used in a building integrated photovoltaic (BIPV) system located in Cyprus. From the methodological point of view, a prospective LCA analysis is usually performed grounding on some foreground scenarios defined by the analyst. In this study, three scenarios regarding the evolution of PSCs operational parameters are considered: a pessimistic, a realistic and an optimistic one. Moreover, an industrial-scale production line for PSCs is modelled by processing primary data retrieved from an existing laboratory-scale process. The LCA model is developed using Ecoinvent 3.7: this database has been merged with a superstructure, built as an excel file, uploaded in the open-source software Activity-Browser that allows to create background scenarios estimating the future changes of Ecoinvent processes. For example, the future national electricity mixes will be strongly different in long-term scenarios. The methodology described in the previous paragraphs allows to calculate the environmental footprint of a BIPV system constructed in Belgium and installed in Cyprus between 2020 and 2060 compared with the electricity mix of Cyprus. The results show that at the current status, the BIPV system equipped with PSCs has a high impact in terms of Global Warming Potential. However, the energy produced by the BIPV systems installed between 2025 and 2040 will be much more sustainable than the grid electricity. This evidence results from a higher maturity of PSCs and from the consumption of more sustainable energy during the construction.

5.05.P-Th157 Applying a Systematic Scenario Methodology Within Prospective LCA: A CASE Study of PHA Production

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PHA production from carbon-rich waste streams by mixed microbial cultures (MMC) can diminish both environmental impacts and production costs. However, these processes are still at low technology readiness level (TRL 5-6). At this level there are still opportunities for major adjustments. Prospective life cycle assessment (pLCA) can be a useful tool to ensure that environmental guidance is included in the development of PHA production by MMC. This research intends to provide insights on the systematic scenario methodology within pLCA of a biobased emerging technology, such as PHA production by MMC. A 4-step process was followed to build the scenarios. The (1) identification of influencing parameters was carried out by applying a PESTEL analysis and causal loop diagram (CLD), where parameter influence and correlations are depicted. After that, (2) sub-scenarios for each parameter under different narratives were constructed. The (3) creation of consistent scenarios from sub-scenarios was carried out through a morphological field analysis. Finally, (4) these scenarios were implemented in the life cycle inventory model. In step (1), an in-depth review of PHA production, which included meetings with technology developers, was carried out to identify the influencing parameters, namely the bioeconomy and environmental policies, type of feedstock, scale and process performance parameters. A hybrid CLD, which describes the influences and correlations between these parameters and the flow chart model, was developed. The construction of sub-scenarios (2) and the upscaling was carried out based on advanced process calculations and pilot-scale data. Consistency check allowed verifying which combination of sub-scenarios for each parameter are most likely to happen. Through the morphological analysis, 4 different scenarios were created: (1) a small-scale on-site production plant within a juice beverage facility under "business as usual" bioeconomy policies, (2) a large-scale production plant that uses fruit waste as feedstock under ambitious bioeconomy policies, (3) and (4) a large scale on-site production plant within an urban waste

and wastewater biorefinery under ambitious bioeconomy policies and low (3) and high (4) process performance parameters respectively. This work demonstrates the utility of combining a systematic scenario methodology with prospective LCA, providing insights of how this emerging biobased process can be developed in a future framework.

5.05.P-Th158 Developing Industrial-Scale Inventories of Energy-Intensive Processes for Bio-Based Activated Carbon Manufacture and Comparison With Data at the Laboratory-Scale

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Using the life cycle assessment (LCA) to evaluate the environmental impacts of emerging technologies faces challenges in collecting the data needed to carry out the analysis. Inputs of materials and energy and outputs of emissions are not readily available or cannot be easily estimated and scaled-up to resemble industrial operations which is necessary to adequately evaluate impacts of these systems and facilitate comparison with concurrent incumbent systems. The current study demonstrates how the aforementioned challenges are addressed in assessment of bio-based activated carbon synthesis. The study showcases how the challenges to data upscaling can be overcome for the processes of activation and carbonization of biochar, and how derived estimates compare to energy consumptions measured in the laboratory settings. The calculation of energy for carbonization and activation involves approximation of several energy flows including the energy consumed to heat the biomass, energy released from the biomass and output gasses, and energy losses from the reactor and output gasses. To quantify these multiple flows we combine information from secondary literature with stoichiometry and fluid dynamics calculations. The laboratory-scale inventory is measured directly or approximated taking into account the power consumption of the equipment. The results demonstrate the approach involving several techniques to approximate industrial-scale data for carbonization and activation processes that are then compared with energy collected at the laboratory scale. Comparison is carried out both directly and at the level of final LCA results. Therefore, the study offers an interesting showcase into dealing with data collection amidst significant gaps and scale-up challenges as one a major bottleneck in the assessment of emerging technologies in LCA.

5.05.P-Th159 Environmental Learning Curves and Their Prospective Applications

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Emerging technologies tend to have a higher cost, lower efficiency and larger environmental impact when compared to an incumbent, which puts them at a disadvantage. This is because incumbents already underwent several iterations of optimisation. Prospective life cycle assessment (pLCA) aims to take into account the optimisation processes to which the emerging technology will be subjected. This makes hotspot analysis of the emerging technology and comparison to the incumbent more informative. One of the methods that is increasingly proposed, but has not seen much development for pLCA application yet, is the application of the learning curve concept. Environmental learning curves (ELCs) are based on the assumption that the environmental impact of a process correlates to how much one has learned about this process and that this correlation can be described with a power law. The ELC describes what happens to the environmental impact of an emerging technology when one increases the “amount of learning”, e.g. by producing more units or installing more production capacity. When a projection is available for the learning parameter at a future point in time (e.g., “by 2030, cumulative production is projected to be 1 million units”), the environmental impact at this future point in time can easily be extrapolated through the ELC. Such straightforward extrapolation is the reason why ELCs are considered the way forward in pLCA of emerging technologies. However, key challenge in application of ELCs for assessing emerging technologies is the reliance on observations and available data in current approaches. Application of ELCs is particularly challenging when assessing emerging technologies, due to absence of historic data and few reported environmental learning rates available. The goal of this study is therefore to present how ELCs can be applied in pLCA to project environmental impacts of emerging technologies at a future point in time. For materials that go into an emerging technology, simple one-factor ELCs are constructed with empirical data using Wright’s law and these individual ELCs are subsequently combined into a component-based ELC for the emerging technology. An example for electricity consumption in primary aluminium production shows that the learning effect on the component level can be substantial, with greenhouse gas footprints projected to reduce by 13% when the energy intensity of the process is subjected to the learning effect.

5.05.P-Th161 Prospective Assessment in the Search for Optimised Pathways for Valorisation of Side Streams in Paper Industry

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With imminent threat from climate change there is an urgent need in transition to circular and sustainable production systems. However, the question remains how do we assess future environmental sustainability of a system that is of low technology readiness level in a robust way? The question becomes even more complex when considering chemicals production systems due to variability of potential production pathways and chemical feedstocks for the same target molecules. Therefore, to analyse and compare environmental and economic effects of different emerging production systems (aiming at the same chemical) in the absence of robust scale-up data, it is necessary to integrate life cycle assessment (LCA) with process modelling: this allows for scale up and optimisation of important production steps (such as separation and purification) before feeding the data into an LCA model. We illustrate this approach on a topical problem of selecting the optimal bio-resource and the production technology to reach a key intermediate molecule. Industrial streams containing bio-feedstocks are attractive potential sources of functionalised molecules, that can enter chemical supply chain as higher value intermediates, rather than be used for energy generation.

Specifically, in the case of the paper industry side streams, terpenes contained in crude sulphate turpentine (CST) and waste streams from other wood pulping processes, could be used as a starting point in the synthesis of a broad range of functional molecules. Bio-feedstocks, however, are not clean and are characterised by significant variability and, often, heterogeneity. Frequently, there may be more than one possible choice for the bio-feedstock source of the molecules of interest. Therefore, identification of the most promising chemical route in early stage process development should, ideally, be accompanied by the evaluation of the combined system of a feedstock and the relevant purification technology options, as a more holistic analysis. Thus, integration of process modelling for generating technological scenarios with life cycle assessment (LCA) for subsequent evaluation of environmental impacts and costing is the most appropriate method of evaluating this holistic problem. In this paper we *integrated process modelling approach into comparative LCA of production of an intermediate molecule nopinone from two alternative paper industry streams containing terpenes, with the aim to identify optimal production route.*

5.05.P-Th162 Prospective Life Cycle Assessment of an Anion Exchange Membrane Water Electrolysis

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Hydrogen is being discussed as a future energy carrier that will play an important role in a future climate-friendly and decarbonized energy system. Particularly water electrolysis is considered a key technology in this transition process, as this technology enables the production of hydrogen by electrochemically splitting water with electricity, preferably from renewable energy for environmental reasons. There are essentially three different water electrolysis technologies. While the solid oxide electrolysis (SOEL) operates at very high temperatures, the alkaline electrolysis (AEL) and the proton exchange membrane electrolysis (PEMEL) represent two low-temperature technologies that are already commercially available. Considering low-temperature electrolysis, the anion exchange membrane electrolysis (AEMEL) currently attracts wide attention. Similar to the AEL technology, the AEMEL operates under an alkaline environment that enables the use of platinum-group-free, non-noble catalysts thus enabling the use of cheap and abundant materials. The integration of a non-porous membrane, as in PEMEL, allows the AEMEL to be fed with pure water and reduces the Ohmic losses facilitating higher current densities and, thus, more compact electrolysis plants. However, the AEMEL technology is at an early stage of development. This study evaluates the environmental performance of an AEMEL using a prospective life cycle assessment, which aims at assessing the environmental impacts of emerging technologies in a more distant future when the technology has achieved higher maturity comparable to that of the incumbent technologies. In this case, the incumbent technologies are the AEL and the PEMEL. The advantage of assessing environmental impacts at a low level of maturity is that at this stage, development can be directed toward good environmental performance. For electrolysis technologies, among the different impact categories, the global warming potential and the use of critical raw materials are often discussed in more detail. This pLCA study shows the future environmental performance of AEMEL compared to the AEL and PEMEL technology and gives an outlook on the future perspective of hydrogen production.

5.05.P-Th163 Life Cycle Assessment Applied to Carbon Capture Technologies for Biohydrogen Production From Waste

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This research work focuses on a complex product system that a) produces hydrogen as the main product b) captures and permanently sequesters carbon dioxide c) utilises biomass waste feedstocks, thereby diverting its fate from landfill or incineration and d) generates electricity as a co-product. This research investigates the environmental performance of carbon capture technologies for the Biomass-to-Biohydrogen process (Bioenergy with CCS or BECCS) by employing a combination of process engineering simulations, technology expert knowledge, commercial-scale proxy technologies as well as lab-scale studies. An LCA was conducted on a complete commercial scale plant converting 110,000 tonnes per annum of waste wood to 50 MWh of grid-quality hydrogen with a focus on four conventional carbon capture technologies, namely, amine scrubbing via monoethanolamine (MEA), Benfield, Selexol® and Rectisol® and an additional emerging technology, Sorption Enhanced Water Gas Shift (SEWGS). Extensive analysis was conducted on the effects of changing system boundaries and functional units, yielding varying results. However, results pointed to a common ranking amongst technologies with respect to climate change category; SEWGS outperforming other technologies, and MEA and Benfield, chemical adsorption-based technologies, performing the poorest. Among the physical adsorption technologies using solvent, Selexol fared better than Rectisol. All other impact categories were analysed, yielding a similar ranking. The work highlights the importance of BECCS as a negative emissions technology and the further environmental benefits of using a physical adsorption technology for carbon capture, particularly SEWGS. This research work provides a real-world application of LCA to a BECCS system producing Biohydrogen from waste and addresses some methodological complexities of prospective LCAs in order to progress from pilot/demo scale towards commercial scale production.

5.05.P-Th164 Prospective Life Cycle Assessment of Carbon Capture and Utilization by Artificial Photosynthesis

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To avoid further risk increases by not exceeding the 1.5°C global warming level, the Intergovernmental Panel on Climate Change (IPCC) advises to reach net negative CO₂ emissions after 2050 according to the SSP1-1.9 scenario. Hence, new technologies for large-scale CO₂ removal will be necessary applying Carbon Capture Storage and Carbon Capture Utilization. However, ensuring

permanent negative CO₂ emissions and in the same time not critically increasing other environmental impacts remains a challenge. An emerging technology which could fill in the gap between carbon dioxide capturing and carbon dioxide utilization or storage, is Artificial Photosynthesis and particularly the photocatalytic reduction of CO₂ to value-added chemicals. Thereof, the utilization path inheres the further advantage of avoiding the impacts related to the conventional production of these chemicals, while in the same time using CO₂ from the ambient air or biomass which potentially leads to net negative CO₂ emissions. However, even if the lowering of CO₂ emissions can be ensured, the widespread application of renewable technologies leads to an increase in land use and material demand due to their dependence on the use of highly dissipated energy. Therefore, it remains necessary to assess the environmental performance of photocatalytic CO₂ reduction regarding its climate change impacts as well as other environmental categories and additionally taking into consideration the very low Technological Readiness level of this technology. Hence, we apply a prospective Life Cycle Assessment of an emerging technology with background data exchange using Integrated Assessment Model scenarios to allow a first estimate of the technology's performance within potential future technospheres. On a system level, we compare two different CO₂ scenarios for the photocatalysis and two different photocatalytic pathways: the photocatalytic CO₂ reduction with a modified parabolic trough collector as solar reactor and the photoelectrocatalytic CO₂ reduction assuming a reactor inspired by photovoltaic technology. We investigate the production of synthesis gas, hydrogen peroxide and methane as promising by-products of the carbon dioxide utilising Artificial Photosynthesis system and compare these to the carbon dioxide capturing and geological storage by Direct Air Capture. The results give a first estimate of the CO₂ removing performance of possible Artificial Photosynthesis systems as well as its performance considering potentially conflictive environmental categories such as land and resource use. Thereof, first conclusions and recommendations about the cross-technology performance as well as optimisation demands and potentials within the emerging technology are given.

5.05.P-Th165 Prospective Life Cycle Assessment on Lab Scale Direct Air Capture System

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To combat global warming, various direct air capture (DAC) technologies are in development to extract CO₂ from the atmosphere. DAC technologies are still in a relatively early stage of development and thereby highly expensive to implement on a large scale. The start-up company Carbyon is currently developing a new technology to remove CO₂ from the atmosphere that can reduce costs by a factor of 10. This technology can adsorb and regenerate CO₂ within a cycle of 60 seconds, i.e. as fast swing process. However, the question remains what the net CO₂ gain is from this new technology and whether that CO₂ profit can still be optimized in the further development of this technology. The goal of this research is to provide recommendations to optimize the net CO₂ gain from direct air capture (DAC) via the new Carbyon process. With a prospective life cycle assessment, the environmental benefits and impacts of the Carbyon DAC system on industrial scale are determined, with focus on how the electricity is supplied and how the captured CO₂ is either utilized or stored. Specifically, climate change, land and water use as environmental impacts are included. The environmental performance of the Carbyon DAC system are also compared with existing DAC technologies.

5.05.P-Th166 Life Cycle Assessment Comparison of Large Scale Hydrogen Delivery Options

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Green hydrogen is expected to play an important role in European decarbonisation efforts. A previous JRC study showed that sourcing hydrogen from a location where renewable energy is cheaper, can be more cost effective than producing it locally. However, the environmental impact of transporting large amounts of hydrogen over long distances, has not been fully understood yet. Our work aims at advancing this field by comparing the life cycle greenhouse gas emissions of three options for delivering hydrogen: hydrogen compression, liquefaction, and chemical bonding to other molecules (i.e., using ammonia, methanol, or liquid organic compounds as hydrogen carriers). The goal is to understand whether transporting hydrogen could make sense from a climate change perspective, and if so, which is the option with the lowest global warming impact. Emissions are assessed from hydrogen production, through its conversion into a suitable carrier, and finally to the shipping and supply of pure hydrogen to an industrial user. A distance compatible with the European territory was considered: i.e., production in Portugal and end-use in the Netherlands. Preliminary results show that all the delivery options would guarantee a supply of hydrogen with a lower global warming potential than electrolytic hydrogen produced from local grid electricity. This is not expected to change within the next ten years, since most European grid mixes will still partially rely on fossil fuels (carbon intensity of the EU grid in 2030 assumed to be 150 g CO₂e/kWh). However, depending on the efficiency of renewable electricity generation and on the source of energy used along the supply chain, shipped green hydrogen could generate more greenhouse gas emissions than local hydrogen produced via steam methane reforming. This is not valid for 2030, when the life cycle of 1 kg of shipped green hydrogen is expected to emit approximately 2 kg of CO₂e. Liquefaction appears to be the least greenhouse gas intense delivery solution, thanks to the lower volumes transported and the lack of an energy intensive transformation process at the delivery site. Nevertheless, much of the Infrastructure for large scale hydrogen delivery does not yet exist, and therefore assumptions on technologies and emissions are subject to a high degree of uncertainty. More primary data on hydrogen technologies and alternative delivery options (e.g., dedicated pipelines) are necessary to increase the accuracy of our estimates.

5.05.P-Th167 eSCALED: A Collaborative Approach for Studying the Life Cycle Analysis of the Components and Assembly of an Artificial Leaf for Renewable Hydrogen Production and CO₂ Utilisation Using Novel Molecular Catalysts and Materials

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The current transition to sustainable energy sources is leading to the creation of new environmental policies. Together with these policies comes the growing importance of objectively quantifying the environmental impacts of emerging sustainable technologies. Artificial photosynthesis is one of these emerging technologies as it is considered a suitable replacement for fossil fuels. Inspired by nature, an artificial leaf produces H₂ or hydrocarbons by converting water and CO₂ using solar energy. However, its implementation in a real life scenario is quite complex. The materials, synthesis procedures, equipment that are required raise issues regarding the sustainability of such energy source. As example, the main reactions in an artificial leaf, oxygen and hydrogen evolution, usually require electrocatalysts based on noble metals such as iridium, ruthenium and platinum. The eSCALED project seeks a collaborative approach to Life Cycle Assessment (LCA) to generate a compilation and evaluation of the inputs, outputs, and potential environmental impacts. This data will then be used to generate a better understanding of the environmental implications and net benefits of manufacture and use of this technology, with each researcher modelling each novel component of the device, such as the electrodes for water oxidation, hydrogen production, electrodes for catalytic carbon dioxide reduction, proton conducting membrane, multijunction solar cells and their assembly into the final device to get a measure of the performance and efficacy of the device as a whole. During this stage, the *primary energy requirement*, that includes all energies related to the extraction of natural resources, preparation of upstream materials and each step required to manufacture the device. The environmental cost for each component part of the artificial photosynthesis device will be simulated using a cradle-to-gate approach to individually analyse the environmental implications of each stage and identify the stages where the highest environmental impact and provide a full explanation of the net benefits and opportunity costs of using this technology. Secondly, a techno-economic analysis focused on the externalities were performed to estimate the costs per kg of hydrogen. The final aim is to give an overview on the environmental and economic aspects of an artificial leaf to have a more informed view on the possible up-scaling this technology for wider use in the energy sector.

5.05.P-Th169 Life Cycle Assessment of an Industrial Vanadium Flow Battery - Identification of Environmental Potentials

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Energy storage systems are becoming increasingly important for a successful energy revolution and will therefore become the focus of attention in the future. Not only renewable energies have to be analyzed for efficiency and sustainability, but also the environmental impact of intermediate storage systems has to be examined in and for the future. A particularly promising stationary battery is the vanadium flow battery (VFB), because of its unique properties for industrial applications. Most outstanding are the independent scalability of the battery's capacity and power, as well as its non-flammability. The VFB offers many advantages to store the energy efficiently and with low emissions. The VFB can run an above-average number of charge/discharge cycles while temporarily storing the energy almost loss-free over a long period of time. In flow batteries, the energy is stored in an electrolyte in chemical energy form, this electrolyte has a significant share in the emissions of a VFB. The basis of the electrolyte is vanadium, which is found primarily in small amounts in iron ores and is therefore responsible for a large proportion of the electrolyte's emissions. In the future, technological advances may prevent most of the emissions from the electrolyte. This development is examined in a prospective LCA of this emerging technology based on the two most important parameters. First, the electrolyte can be processed with little effort and thus can theoretically be used indefinitely. Another point is the processing of the iron ore respectively the steel production with green hydrogen, whereby the vanadium as a by-product causes significantly less emissions. The basis of the study is the modeling of vanadium pentoxides using primary data from a mine in South Africa, based on the data, the correlation of the parameters and their effects is evaluated in a global sensitivity analysis. The emissions and future potential of batteries have only been analyzed to a limited extent to date. No dependence on critical raw materials exist due to these new approaches and likewise, the VFB is a particularly sustainable battery. In politics and economics, the VFB is often not taken into account due to the cost of the system. The two parameters presented have a significant impact on the reduction of emissions, but also the reuse leads to a significant reduction of costs.

5.05.P-Th170 Prospective Life Cycle Assessment of a Sodium-Ion Battery Focusing on Climate and Resource Impacts

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Rechargeable batteries are increasingly used in a number of applications, such as consumer electronics, electric vehicles, and stationary energy storage. An increased use in the latter two applications is envisioned to reduce greenhouse gas emissions. However, the dominant rechargeable battery technology – the lithium-ion battery (LIB) – impacts the environment in several ways throughout its life cycle. In addition, LIBs require critical and/or geochemically scarce materials, such as lithium, natural graphite, and sometimes nickel and cobalt. One promising next generation battery (NGB) is the sodium-ion battery (SIB). While other NGBs can provide higher energy densities, the SIB technology holds great promise from a resource point of view, since it can be made to contain mostly low-cost, abundant and readily available elements, such as sodium and iron. In addition, the manufacturing processes and equipment developed for LIBs can in principle be re-used, enabling convenient scale-up of production. We here assess the life-cycle impacts of a specific SIB with a low content of scarce metals using prospective life cycle

assessment (LCA). The SIB is assumed to be a mature technology produced at large scale and this we accomplish by using data from a small-scale producer and scale these up using available large-scale factory data for LIB production. We use a functional unit of 1 kWh of installed battery cell storage capacity and focus on climate and mineral resource impacts, since those have been highlighted in several publications and guidance documents as particularly important to address in LCAs of batteries. Different shares of renewables are considered in energy supply scenarios, along with scenarios for specific energy density developments. The impacts are compared to those of large-scale produced LIBs and to another NGB – the lithium-sulfur battery. To investigate mineral resource impacts of the different technologies in depth, we include two resource impact assessment methods, the crustal scarcity indicator and the surplus ore potential. The aims of the study are (i) to assess the prospective life cycle impacts of the SIB technology in order to reveal whether it is preferable to other battery technologies from an environmental and resource point of view, and (ii) to understand the environmental profile of the SIB in order to identify hotspots.

5.05.P-Th171 Prospective Life Cycle Assessment of the Electricity-Based Primary Steel Production Technology of Electrowinning

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It is crucial to decarbonize steel production systems to achieve the climate targets of the Paris Agreement, as steel production is currently responsible for 7 to 9% of global greenhouse gas emissions. Most of these emissions are caused by primary steel production, specifically the most commonly used route using a blast furnace (BF) and basic oxygen furnace (BOF). The BF-BOF route is strongly dependent on coal, as it allows to achieve the very high temperatures required and also serves as a reducing agent. Low-emission, cost-competitive and ready-to-implement alternatives are currently lacking, which makes the steel sector hard to decarbonize. A key strategy for decarbonization is electrification. In the case of steel, electricity-based technologies for primary production, i.e., electrolysis of iron ore, are still under development. Electrolysis of iron ore at low temperatures (~110°C) can be realized through electrowinning (EW), an emerging technology with a technology readiness level of 4 to 6. First, this study aims to determine the future environmental impacts of primary steel production via electrowinning (EW) using prospective life cycle assessment (LCA). Second, we compare the environmental performance of EW with the incumbent production route of BF-BOF. We developed a life cycle inventory for primary steel production via EW using data from literature and the background database ofecoinvent. Data gaps have been filled using assumptions, e.g., based on stoichiometric relations or expert judgement. Impacts are calculated for 1 kg of steel produced in Europe for the time period of 2020 to 2050. To account for transitions in the background system, we integrated background scenarios for electricity supply of global coverage from integrated assessment models. Lastly, the future environmental impacts of EW are compared to the conventional route of BF-BOF, which is modelled with datasets from ecoinvent. The results show that EW reduces direct emissions from steel production compared to the BF-BOF. Yet, considerable impacts still occur upstream of steel production processes, specifically during electricity production. Thus, the environmental benefit of EW strongly depends on the environmental performance of electricity supply. This stresses the relevance of taking a systems perspective and of background scenarios which allow to consider scenarios also in sectors other than the foreground system.

5.05.P-Th172 LCA for Sustainable Innovation: A Prospective View on Printed Circuit Boards vs. Printed Electronics

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Novel Printed Electronics are manufactured through additive methods and are proposed as sustainable alternatives to conventional Printed Circuit Boards (PCBs), especially in novel and niche Internet-of-Things (IoT) applications. Thus, this study aims to verify the environmental advantage of paper-based printed electronics through a comparative LCA with PCBs. The LCA considers the flow of conductor and substrate materials through the various phases of an optimistic lifecycle (including recycling) of PCBs and printed electronics. All the relevant processes throughout the lifecycle of printed electronics and PCBs have been modelled in a life cycle inventory (LCI) using Ecoinvent v3.7.1 and the environmental impacts were calculated as per ILCD's 2018 total climate change method. The results highlight that the Silver (Ag) ink-based conductor used in printed electronics has a higher net environmental impact over the entire lifecycle than the Copper (Cu) conductive tracks of the PCBs; this is attributable to the higher environmental impact of Ag and chemicals required to manufacture the conductive ink. Furthermore, from a substrate perspective, the PCBs glass-fibre and epoxy resin-based FR4 boards have a much higher net impact over the entire life cycle than the CNC-coated paper substrate required for the printed electronics. Combining the impacts of the conductor and substrate for a conclusive comparison between PCBs and printed electronics underscores that the substrate material is crucial: the lower impacts from the CNC-coated paper give printed electronics an environmental advantage over PCBs. Thus, further exploration of replacement possibilities for PCBs with printed electronics is viable from a sustainability perspective. This study also exemplifies how LCAs are critical in identifying and solving environmental challenges associated with innovations. As a decision-making tool, an early stage LCA can verify the sustainability prospects of a project, and assist in targeting and remedying sustainability hotspots early on while the material and product development process is still flexible and open to adaptations.

5.05.P-Th173 Development of a Radiative Cooling Material Database to Benchmark the Environmental Impact of a Newly Developed Photonic Meta-Concrete

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Global warming and the heat-island-effect in cities led to an increased use of conventional air-conditioners. This increased usage is responsible for 10% of the total energy consumption and 7% of global greenhouse gas emissions. In the overall aim to reduce

these greenhouse gas emissions and to mitigate climate change and the heat-island-effect, a new radiative cooling material is under development. Although radiative cooling materials already exist, this material stands out because, for the first time, it is based on conventional concrete. This paper describes the first step of the environmental life cycle assessment (LCA) study that is performed along the development of the photonic meta-concrete (PMC). In this step we generate a database with environmental data of existing radiative cooling materials in order to create a comparative base for the PMC. Information about the various materials is collected through a literature review and consultation of experts. The environmental impact of the materials are assessed with the Belgian LCA method for buildings, i.e. the MMG method. The MMG method is in line with the EN15804:A2 and hence covers 16 environmental impact categories, such as global warming potential, acidification, eutrophication, water scarcity, toxicity. Furthermore, the environmental impact will be compared with the first composition of the photonic meta-concrete. The first results show that a lot of product-specific information regarding radiative cooling materials is lacking to date to assess their environmental impact and this makes it challenging to create a reference database. However, the environmental impact of the radiative cooling materials assessed in this paper is in line with the expectations. The first mixture of the photonic meta-concrete is promising as this shows great potential from an environmental point of view compared to the existing daytime radiative cooling materials.

5.05.P-Th174 Prospective Life Cycle Assessment of Road Infrastructure in the Context of Circular Economy: Advances, Challenges, and Opportunities

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Road infrastructure is an integral building block of our society by provisioning essential services (e.g., movement of resources and commuting) and stimulating the socio-economic development of local communities. The deterioration of road infrastructure is a dynamic process that affects such essential services through degrading the pavement durability and increasing the rolling resistance, which further influences the material and energy consumption for pavement maintenance, rehabilitation, and reconstruction. Existing life cycle assessment (LCA) studies of pavements have established a foundational framework to estimate environmental impacts but fail to include the dynamics of pavement in the system. In response to calls for a transition toward a circular economy, utilization of secondary materials through various recycling technologies (e.g., hot, warm, or cold recycling) has been increasingly considered in the LCA of road infrastructure. However, it remains unclear under what conditions one recycling technology is superior to other options. In order to understand the key methodological advances and challenges and identify opportunities for improving the utility of LCA tools of pavement, we provide a critical review of recent pavement LCA literature by conducting a semi-quantitative analysis on geographic coverage, temporal trend, time horizon, functional unit, life cycle stages, and consideration of reclaimed/secondary materials. In this review, we reflect on a variety of methodological and data issues, including inconsistencies in the functional unit, ambiguity of system boundary, incompleteness of certain life cycle stages, and omission of certain important impact categories, but also highlights that due attention to the use phase of pavement can improve the utility of pavement LCA significantly. Our review finds that using more secondary materials (e.g., reclaimed asphalt pavement, recycled tire, waste glass, construction & demolish waste, etc.) in pavements can reduce environmental impacts notably. For future research, it is recommended that knowledge gaps in the use phase of pavement, such as albedo effect, rolling resistance, and lighting, should be addressed in pavement LCAs, and more sophisticated models (e.g., mechanistic-empirical models) should be used to predict the deterioration of pavement performance to have accurate LCA results and close-to-reality estimation of location-specific effects.

5.05.P-Th175 Life Cycle Assessment of Commercial Scale Ozonation Plant for Advance Oxidation of Micropollutats in Wastewater Treatment. Case of Study in Netherlands

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The improvement of water quality and ecosystems are important UN goals towards sustainable development. In Europe, effluent discharges from wastewater treatment plants are the main cause of degradation of ecosystems, due to the presence of micropollutants. These compounds, which cover a wide range of chemical species of organic nature, are poorly degraded in conventional wastewater treatment, and their presence in water bodies remains at low concentrations ($\mu\text{g/l}$) causing negative effects due to chronic exposure. Thus, an improvement in conventional wastewater treatment plants is becoming urgent to ensure the efficient removal and safeguard the protection of water resources. Ozonation has amply proven its effectiveness in disinfection of drinking water, due to the high oxidative power of the ozone molecule, able to react with a large number of organic compounds. For this reason, this advance oxidation technology can also be applied to the degradation of recalcitrant micropollutants in wastewater streams, whose large-scale implementation as a tertiary treatment of wastewater effluents is becoming more and more attractive. Hence, it is crucial to take into account the environmental burdens involved in the deployment and implementation of commercial scale of ozonation as tertiary treatment. In this study, Life Cycle Assessment is used to analyse the environmental performance of ozonation as an emerging technology for the advance oxidation of micropollutants from wastewater effluents in tertiary treatment of municipal wastewater. A commercial scale Ozonation facility developed in the Netherlands has been chosen as a case of study. The functional unit is the treatment of an average effluent flow of 580m³/h, with a dosage of ozone of 0.7gO₃/gDOC, for the removal of 70% of the micropollutant load from the wastewater effluent. The system boundaries comprise a gate-to-gate study, focusing on the use phase of the ozonation system, which entails in situ ozone generation and effluent treatment in the ozone reactor. Results show that the main environmental burdens are attained to energy consumption, mainly associated to the in situ ozone generation, followed by the pumping of the effluent from the treatment plant to the reactor. This study demonstrates the relevance of LCA as a powerful tool to determine the sustainability of

early stage implementation technologies in wastewater treatment. Allowing to refine the decision making-process for its large-scale consolidation.

5.05.P-Th176 Comparing Prospective Methods for Life Cycle Assessment of Emerging Energy Technologies

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The introduction of new energy technologies that intend to produce lower environmental impacts is one option to tackle climate change. However, it is still unclear to what extent these technologies can actually contribute to reach the sustainability goals. To answer this question, the amount of resources needed and the potential environmental impacts throughout the whole lifecycle of emerging technologies should be determined. One approach to accomplish this is to follow the well-known life cycle assessment (LCA) methodology. Nevertheless, this scheme is generally intended for assessing mature technologies that have been already introduced into the market. Therefore, a proper framework that considers the inherent challenges of future-oriented assessments including limited data, scalability and uncertainty is still lacking. Consequently, the outcome of our research consists in providing a guideline on choosing methods for conducting a prospective LCA of emerging energy technologies, as well as their link with uncertainty characterization and quantification. For instance, these methods can include: extrapolation, technological learning, technological diffusion, own assumptions, participatory approaches and scenario analysis. To the best of our knowledge, these methods have not yet been extensively explored in the literature. Thus, at this conference, we would like to present the following first steps to this approach: · The identification, comparison, selection and categorization of methods for generating life cycle inventory (LCI) data of emerging technologies at early development stages. · The criteria for the selection of methods in order to be applicable to energy technologies and compatible with uncertainty management. · How these findings can contribute to explore new modeling approaches and to better understand uncertainty in the LCA of technologies under development.

5.05.P-Th177 Environmental Assessment of New Technologies for Mixed Plastic Waste Treatment Using LCA

Methodology

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The poster presents a project in which the concepts of integrated systems for the management of mixed plastics are proposed. These concepts will respond to new trends and principles of waste policy of the European Union, i.e. landfill reduction and, to the maximum extent, material and energy recovery of waste and overall environmental acceptability. The subject of the Life Cycle Assessment are two new technologies for the treatment of mixed plastic waste, which are promising and will enable the further development of the circular economy. These are technologies for thermal decomposition of plastic waste and Polybet. Thermal depolymerisation is a method of energy recovery of carbon-based materials. It is a physicochemical endothermic process that occurs in an anaerobic environment at various stages at temperatures ranging from 300 to 2 000 °C. These temperatures exceed the limits of thermal and chemical stability of organic and synthetic compounds, they decompose and form low molecular weight products and solid residue. The input materials will be mixed plastic waste produced as residual fractions in production processes. Thermal depolymerisation cleaves hydrocarbon chains and releases relatively low molecular weight process products. These process products (process oil and process gas) will be used as fuel in the production process (gas) and as a raw material for further processing (oil). The inorganic inert material (carbon) will remain relatively unchanged as a solid residue after depolymerisation and will be further recycled for further practical use. Polybet technology recovers mixed thermoplastics and internal fillers. The resulting composite has widely usable technical properties, especially minimal water absorption. It is a technology for a preparation of polymer concrete mixture with high variability of inputs and a wide range of applicable recipes. The final shape of the final product is designed individually according to the specific requirements of the customer. It is expected to produce a universally usable construction product of medium dimensions, such as road curbs, blocks, footings or tiles, or variants of partition walls. The project also proposes a detailed methodology for assessing these types of technologies using the LCA method in order to ensure an independent and realistic environmental impact assessment.

5.05.P-Th178 Conceptualizing Prospective LCA Approaches to Assess Novel Perovskite Photovoltaic Technology at Lower TRLs - the CASE of the H2020 APOLO Project

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Prospective assessment approaches to LCA on emerging technologies, such as perovskite solar cells (PSC), have been argued to offer more informative and relevant impact results, especially when attempting comparisons with mature technologies. However, few if any European Commission-funded research projects evolving around perovskite technology seem to apply a prospective assessment approach to their LCA that considers the potential impacts of full-scale implementation at a future point in time. The H2020 APOLO is developing fully printable perovskite solar cells on flexible substrates, which are to be integrated in varying formats into different demonstrators for architectural uses in building and structures, combining functionality with aesthetics. With the purpose of supporting the R&D stage from an environmental and eco-design perspective, the project contains a LCA that, in so far as possible, considers likely industrial production means, and makes projections about potential use, re-use, and end-of-life scenarios, thereby attempting to step beyond the limitations of actual lab-scale and demo-focused production taking place under the umbrella of the project. Building on existing literature and frameworks, as well as actual impact results, the example of the APOLO project serves to discuss which life cycle stages and underlying aspects may be most susceptible to future changes and uncertainty compared to the status quo, and where major opportunities and necessities may lie for the application of a more comprehensive and effective prospective assessment in future PSC or other PV technology LCA research.

5.05.P-Th179 Life Cycle Assessment of Highly Emissive CsPbBr₃ Perovskite Nanocrystals

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Despite being a small contributor to the current global electricity production, interest in solar cells has rapidly increased over the past decade, and photovoltaic (PV) industry is expected to have major role in the electricity market in the long term. High power conversion efficiency, low costs of materials and simple and eco-friendly manufacturing are main requirements for solar cells. Promising candidate in such search are perovskite solar cells. Perovskite is a term used for the whole group of organic-inorganic materials with generic formula ABX₃ and crystallographic structure based on a structure of calcium titanium oxide. Perovskite nanocrystals are able to act as semiconductor nanocrystals with an exceptional photophysical (photoluminescence quantum yield and easily tunable photoluminescence emission) and primary optoelectronic properties (direct bandgap, tunability of bandgap, low recombination rates, long carrier diffusion length and high carrier mobility). In addition, its easy processability with the use of wide available chemical components makes them very attractive for mass production. Many synthesis processes for CsPbX₃ were reported among many groups, but CsPbBr₃ NCs in this research were prepared using so called ligand assisted room temperature precipitation (LARP) and more common hot- injection (HI). Environmental impact of perovskite solar cells is very important to determinate, since they are still in a process of development and it is important to estimate its impact for the possible mass production; to that end, a cradle-to-gate LCA study has been performed. Our recent results showed that, for lead halides salts the most contribution in all impact categories is coming from lead. But, for CsPbBr₃ nanocrystals, prepared via LARP, Isopropanol has the most impact and in hot- injection method, Oleylamine. It was of a high interest to compare contributions to emission, for the same type and amount of nanocrystals (1 kg of CaPbBr₃), but produced with already mentioned two different synthetic ways. Perovskite nanocrystals, as itself, do not contribute significantly to the global emission comparing to the other layers in perovskite solar cells, which is positive result for the massive production. From the point of view of the Ecodesign, these LCAs are important to choose and/or redefine the processes to be up-scaled to the industry level.

5.05.P-Th180 Life Cycle Assessment of 3D Printed Furniture Using Fine Recycled Aggregates - Cirmap Project

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In North West Europe, about 65 Mt of Recycled Fine Aggregates (RFA) are generated yearly from the crushing of Concrete Construction and Demolition Wastes and are disposed in landfills or in banks. In the meantime, 54 Mt of marine sands are extracted in zones where natural aggregate resources are missing, threatening fragile marine spaces. Reusing RFA in concrete would save natural resources. However, no market exists for RFA owing to rigorous construction standards. RFA is a local resource, with large variability, which is not suited to mass production needing regular materials. But it could be used at a smaller scale, for the manufacture of products needing neither to comply with rigorous construction standards nor to possess high performances. About 5 Mt of RFA could be recycled into concrete for the manufacture of Urban, Memorial or Garden (UMG) furniture, being the starting point of a circular economy loop. Traditional precast concrete cannot be used for small scale production because of the high share of moulds in the global cost (50 to 80%). However, concrete 3D Printing (3DP) allows manufacturing customized pieces that could be shown by customers as a banner of their identity. UMG furniture are always located in difficult to access zones, reducing their weight by shape optimization and printing them onsite would be easier. Moreover, online control of 3D Printing could be used to compensate materials variability, providing an efficient tool for the valorisation of these resources. Cirmap will provide a new Mixture Proportioning Method (MPM) for the design of 3DP mortars with RFA and a new Design Methodology for Customized Shapes (DMCS). A new Master Control Command (MCC) for concrete 3DP will be implemented for the equipment of 3DP machines, and an integrated mobile 3D printing unit will be developed for onsite 3D printing of UMG furniture. A Cirmap-network will also be created for lobby and dissemination, leading to a new market for the reuse of RFA. The Chemical Engineering research team is in charge of the environmental aspects of the project and applies Life Cycle Assessment (LCA) in an eco-design approach. Acknowledgements - The authors would like to thank the Cirmap project (INTERREG NWE, n° NWE1062), partly financed by the European Regional Development Funds, and the Walloon Region.

5.05.P-Th181 Handling Multifunctionality Within a Prospective LCA of a Biobased Emerging Process: A CASE Study of PHA Production

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PHA production from carbon-rich waste streams by mixed microbial cultures (MMC) can diminish both environmental impacts and production costs, specially when it is integrated into a biorefinery approach. However, these processes are still at low technology readiness level (TRL 5-6). At this level there are still opportunities for major adjustments. Prospective life cycle assessment (pLCA) can be a useful tool to ensure that environmental guidance is included in the development of PHA production by MMC. Under this research, different approaches on how to handle multifunctionality within a prospective LCA of PHA production are discussed. PHA production is 3-step process comprised by anaerobic fermentation, biomass selection and PHA accumulation. After that, PHA is recovered from the biomass in the downstream process. Waste streams produced in anaerobic fermentation and downstream processing are sent to anaerobic digestion, where biogas and digestate are produced. Biogas produced in both anaerobic fermentation and digestion are transformed into electricity and heat in a CHP unit. Two different approaches can be applied to solve the multifunctionality: partitioning or substitution. When applying partitioning, system was splitted into 5 different subsystems: (1) anaerobic fermentation, (2) biomass selection and PHA accumulation, (3) PHA

downstream, (4) anaerobic digestion and, (5) cogeneration. Subsystems (1), (3) and (4) functional flows ((1) VFA, biogas and solid-rich waste stream, (3) PHA and waste stream and (4) biogas and digestate) were allocated based on COD, while subsystem (5) functional flows (electricity and heat) were allocated based on exergy. PHA production involves subsystems (1), (2) and (3), while consumed heat and electricity comes from subsystem (5). Regarding the substitution approach, PHA production system is expanded including the waste treatment, anaerobic digestion and cogeneration. Digestate, heat and electricity avoided products are related avoided products are fertilizers, steam production and EU electricity mix. Substitution entails more challenges than partitioning, since requires more data (e.g., digestate composition) and non-identical functional flows will have to be considered equivalent (e.g., electricity mix based on renewables). Furthermore, when applying a systematic scenario methodology, given credits by an avoided product may differ depending on the background parameter (e.g., electricity mix based on carbon source vs renewables).

5.05.P-Th182 From Lab to Plate: Life Cycle Assessment of Valorization of Herring and Lingonberry Co-Products

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With the aim of conserving resources, several methods have been developed to recover protein from fish filleting co-products. These products can be destined for human consumption such as pate, fish burgers, fish sausages, or used as soup thickeners. A recently developed technique has been demonstrated at laboratory scale for the extraction of protein-based on cross-processing of coproducts from herring (*Clupea harengus*) filleting with lingonberry (*Vaccinium vitis-idea*) pressing coproducts. The outputs of the cross-processing are three products: oil, protein, and other solids. We carried out a life cycle assessment of this cross-processing by up-scaling it to an industrial scale using literature data and expert information, considering the volume of the herring and lingonberry co-products available in Sweden. The protein product was modeled as being used in a fish ball with the addition of other agricultural ingredients, and it was compared with a commercially available fish ball. Calculations were run in OpenLCA v3.01 software, using ecoinvent, and Agribalyse data for background process as well as literature and industry data. Environmental impacts were assessed in terms of climate change, energy consumption, land occupation, and marine biotic resources depletion. The results showed that fishery operations and the transportation of both berries and of the berry-picking workforce dominated the impact of the protein product for climate change. The protein product dominated the impact of the fishball in all categories. For land occupation, an improvement potential was associated with using oil derived from the cross-processing itself instead of vegetable oil in the product formulation. Beyond the production of the recovered protein and raw materials, the building infrastructure and production of electricity for chilled storage and processing played significant roles. When assessed as a consumable product, the fishball made with recovered protein represented less environmental impact than the benchmark fish ball in all categories investigated. Overall, the results support the utilization of herring and lingonberry co-product as a source of protein for human consumption, with LCA helping identify an environmental opportunity that can also improve the nutritional profile of the product.

5.06 Safe and Sustainable by Design for chemicals, materials and products

5.06.T-01 Safe and Sustainable by Design: A Computer-Based Approach to Redesign Chemicals for Reduced Environmental Hazards

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Persistency of chemicals in the environment is seen as a pressing issue as it inevitably results in the accumulation of these chemicals over time and the possibility of long-term exposure resulting in adverse effects. Persistent chemicals can on the other hand be an asset in a well-functioning circular economy where products are more durable and can be reused or recycled. However, this objective cannot always be fulfilled as the release of chemicals from products into the environment can be inherently coupled to their use. In these situations, the chemical should be designed for degradation to avoid adverse effects. In this study, an approach was developed to select chemicals for redesign and to facilitate the systematic design of Safe and Sustainable chemicals based on in silico structure generation. The resulting approach includes elements of Green and Circular Chemistry, Essential Use, and Alternatives Assessment and ties into goals recently formulated in the context of the EU Green Deal. We used the organophosphate compound tri-isobutyl phosphate (TiBP) as a case study for exploration of the approach, as the emission of TiBP to the environment was expected to be inevitable for some of its uses as a flame retardant. Over 6.3 million suggested alternative structures for TiBP were created in silico, and then filtered based on outputs from quantitative structure-activity relationships (QSARs) for potentially non-readily biodegradable structures. The remaining structures were further analyzed based on QSAR predicted hazard properties for persistency, bioaccumulation, mobility and toxicity (PBMT) and synthesizability. With a multi-criteria analysis, a top 100 of the most desirable structures was identified from which a target structure (di-n-butyl (2-hydroxyethyl) phosphate) was selected and subsequently synthesized. The first experimental results indicate an enhanced functionality of the alternative compound which would subsequently reduce its environmental impacts. The approach provides a new, systematic and computer-aided workflow for the chemical redesign for reduced persistency and can help take Green and Circular Chemistry principles for the design of Safe and Sustainable chemicals into practice. The approach can be expanded and further verified to reach its full potential in the mitigation of chemical pollution and to help enable a safe circular economy.

5.06.T-02 Can Cable Plastics Be Safe and Sustainable by Design at the End-Of-Life

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Waste of electrical and electronic equipment (WEEE) is one of the fastest growing waste streams in the EU and its toxic components pose a substantial threat to environmental and human health. Cables are one of most plastic rich WEEE items, typically containing 50 % polyvinylchloride (PVC). PVC from electrical cables often contain bisphenols (BPs) as antioxidants to prolong the lifespan of the cables, a group of high volume produced, hazardous compounds with well-known potentials for endocrine disruption in humans and wildlife. Currently, the metals and plastic are separated by granulation, however recycling of cable granulates is only on a business-to-business level. Consequently, most PVC cables and cable granulates are landfilled or incinerated with energy recovery. Yet, energy recovery of PVC is challenging due to the formation and emissions of dioxins, furans, hydrochlorid compounds, and heavy metals during incineration. Consequently, an increasing fraction of cables and PVC cable granulates are being landfilled in Norway, despite that landfilling is the least preferred option of waste management in a circular economy. To evaluate the potential for end-of-life recovery for reuse and/or recycling of cables, the present study aims to investigate the mass flow of BPs in cable plastic in Norway between 2018-2020. To provide information essential for designing safe and sustainable cables, the potential transfer of BPs to plastic recycles and emissions and impacts on the environment was investigated. Our mass flow analyses shows that on average, 38% of the collected cables were deposited onto landfills between 2018 - 2020, of which was 4 900 tonnes cable plastic (incl. fly and bottom ash) containing over 300 kg of BPs which accumulated on the landfill each year. Landfilling was also the major contributor to the environmental emissions with a total of 50 kg BPs emitted as lechate or particles to sludge, water and air each year. Incineration with energy recovery and thermal destruction efficiently removed 130 kg of BPs from the mass flow, however almost 100 kg of BPs was sent to material recycling to produce new products. As such, the present study emphasize a need for designing safe and sustainable plastics where end-of-life waste management is considered, such as by phasing out PVC and BPs and other hazardous substances. Further, it shows the need to improve the current waste management of cables to reduce environmental emissions of BPs.

5.06.T-03 Implementing Safe by Design in Product Development Through Combining Risk Assessment and Life Cycle Assessment

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The Safe by Design (SbD) concept aims to ensure safe, sustainable and circular materials and products from early stages of product development. While there is growing interest in the potential of SbD to help meet the goals EU Green Deal and Circular economy, methodological approaches and practical guidelines on SbD for product design are however largely missing. The combined use of Life Cycle Thinking (LCT) or Life Cycle Assessment (LCA) and Risk Assessment (RA) is considered suitable to operationalize SbD. We explored the potential of the combined use of LCT/LCA and RA by product design teams at Technological Readiness Level (TRL) 1-6 through a literature review combining LCT/LCA and RA at early stages of product design, to identify and understand the approaches that have been used to combine the methods, and the potential of these approaches for product design teams to implement SbD. We found that product design teams can already perform themselves basic early-on-evaluations of safety and sustainability (e.g., apply lifecycle thinking to assess risk hotspots, avoid use of hazardous chemicals, minimize environmental impacts) while collaborating with experts or coordinating across the value chain will be necessary for more complex assessments (ex-ante LCA, control banding, predictive toxicology, etc). The application of these simplified approaches and guidelines cannot substitute a full-fledged RA or LCA at higher TRLs but they may help avoid some obvious sources of risks and impacts early on. However, the full potential of combining these methods has not been exploited in most of the literature. Critical gaps need to be addressed before SbD can be operational in product design practice, including more studies in product design context, developing tools and databases from the product designer's perspective, greater collaboration between RA/LCA researchers and companies, and policy discussion on the expansion from SbD to Safe and Sustainable by Design (SSbD).

5.06.T-04 Deriving Non-Cancer Human Toxicity Effect Factors With Quantified Uncertainty for More Than 10,000 Chemicals

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Life cycle impact assessment (LCIA) characterizes toxicological impacts on human health from chemical exposures via effect factors based on chemical-specific points of departure (PODs) from regulatory sources. However, such data are available only for a few hundred substances, and consequently, effect factors are lacking for the broader range of currently marketed chemicals. To address this gap, recent updates of the globally recommended approach for deriving human dose-response factors for non-cancer endpoints in LCIA propose using experimental animal data to estimate PODs for substances lacking regulatory data. The aim of this study is thus to broaden the coverage of chemicals by using available *in vivo* data to estimate suitable PODs and derive non-cancer health effect factors for application in LCIA. As a starting point, we curated and selected experimental animal toxicity data from the U.S. EPA's Toxicity Value Database following a semi-automated approach and built two distinct datasets covering reproductive/developmental and other non-cancer effects. Next, we fitted the curated data to a lognormal distribution for each chemical in the datasets and derived PODs as the 25th percentile of the fitted distributions to best mimic regulatory data ($R^2 > 0.78$). In addition, we quantified for each POD uncertainty, considering both intra-study and inter-study variability. We then derived human-equivalent lifetime dose-response factors from the identified PODs. Finally, dose-response factors were combined with

severity factors to derive effect factors expressed in disability-adjusted life years lost associated with incremental exposure (DALY/kg_{intake}), differentiating between the two disease categories considered. Based on this approach, we estimated non-cancer health effect factors with quantified uncertainties for 6,703 substances covering reproductive/developmental effects and for 8,032 substances covering other non-cancer effects. The estimated effect factors range across chemicals over several orders of magnitude from 4.4×10^{-5} to 2×10^8 DALY/kg_{intake} with effect factors covering reproductive/developmental effects being on average higher per chemical. These results significantly broaden the coverage of chemicals by estimating effect factors also for substances without regulatory data. Next steps include extending this approach to cover additional exposure routes, such as inhalation and dermal exposure, and increasing the differentiation in non-cancer effects.

5.06 Safe and Sustainable by Design for chemicals, materials and products (Virtual Only)

5.06.V-01 ALBINA: Developing Sustainable, Environmentally Friendly Lubricant Additives for Technical Applications From Algae

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The interdisciplinary project ALBINA aims at formulating new lubricant additives based on biological and renewable algae products for use in technical applications. The project is funded by the German Ministry of Nutrition and Agriculture's Agency for Renewable Resources. Conventional mineral oil based lubricant additives are often highly toxic to humans and the environment. The goal of this project is to find alternatives with algae based sugar and protein mixtures. The development chain starts with the selection and cultivation of the algae, their extraction, their chemical structure analysis and their ecotoxicological characteristics. The technical application in cutting and reshaping metals are tested as well. A wide range of algae species have been cultivated under varying conditions to optimize the output. Extensive methodological research was necessary to develop extraction protocols with different solvents for extracellular polymeric substances and biomass of various algae species. Especially creating oil-based solutions with algae products for technical testing posed a major challenge. Ecotoxicological screening with acute tests at 1 g L^{-1} *Daphnia magna*, *Arthrobacter globiformis*, *Enchtraeus crypticus* and *Folsomia candida* so far have indicated low toxicity of most extracts. So far, five algae species render the most favorable results; therefore chronic daphnia, algae and Collembola tests are currently being conducted to assess potential long-term toxicity. Biodegradation experiments will run with the technically promising candidates that have been identified so far. We would like to present the aim and structure of ALBINA as well as the processes and results of developing sustainable and environmentally friendly lubricant additives.

5.06.V-02 Review of Frameworks for Safe and Sustainable-By-Design Criteria Definition for Chemicals and Materials

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In the context of the EU Green Deal, the Chemicals Strategy for Sustainability (CSS) aims at improving safeguard of human health and the environment as part of an ambitious approach to tackle pollution from all sources and move towards zero-pollution for air, water and soil. A key action defined in the CSS is the development of safe- and sustainable-by-design (SSbD) criteria for chemicals. The SSbD concept promotes a holistic approach that integrates safety, circularity and functionality of chemicals, materials, products and processes throughout their entire lifecycle and minimises their environmental footprint. As a first step for the development of such a framework, we conducted a review of existing safety and sustainability frameworks and the underpinning concepts, methods, models, tools and indicators used to address sustainability dimensions. The starting point of this review was the mapping study and the results of a targeted stakeholders' survey conducted by European Commission's Directorate General Research and Innovation. There are multiple challenges related to the integration of safety and sustainability dimensions (as well as the related concepts) and the review has covered approaches proposed by different stakeholders, being in research, business, government or civil society (e.g. NGOs). A few frameworks encompass safety and the three dimensions of sustainability (environmental, economic and social) and many frameworks are purely conceptual, listing elements to be included but without reporting the operational methods or approaches to address qualitatively or quantitatively the issues at stake. In most of the frameworks safety considerations refers to legislative requirements. Regarding life cycle considerations, these are often mentioned and integrated in the frameworks, with different level of details in terms of models and indicators to be adopted. In some of the framework, criteria are already derived and illustrated, and are complemented by operational tools that allow ranking chemicals based on specific criteria.

5.06 Safe and Sustainable by Design for chemicals, materials and products (Poster)

5.06.P-Tu296 Machine Learning Models Based on MOLECULAR Descriptors to Predict Toxicological and EcoToxicological Characterization Factors

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It is a real challenge for life cycle assessment practitioners to identify all relevant substances contributing to the ecotoxicity. Once

this identification has been made, the lack of corresponding ecotoxicity factors can make the results partial and difficult to interpret. So, it is a real and important challenge to provide ecotoxicity factors for a wide range of compounds. Nevertheless, obtaining such factors using experiments is tedious, time-consuming, and made at a high cost. A modelling method that could predict these factors from easy-to-obtain information on each chemical would be of great value. Here, we present such a method, based on machine learning algorithms, that used molecular descriptors to predict two specific endpoints in continental freshwater for ecotoxicological and human impacts. The different tested machine learning algorithms show good performances on a learning database and the non-linear methods tend to outperform the linear ones. The cluster-then-predict approaches usually show the best performances which suggests that these predicted models must be derived for somewhat similar compounds. The median values of the absolute errors were below one log, which is the commonly assumed margin of error for experimental characterization factors. This promising results show that this methodology could be a quick alternative to provide characterization factors when they are lacking. It could be noticed that this machine learning predictive strategy could be applied to any other compartment and/or characterization factors, provided that a sufficiently large learning database already exists.

5.06.P-Tu297 New Life Cycle Inventories of Chemicals Used in the Production of Airframe Parts

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New Life Cycle Inventories (LCI) of chemicals used in the production of airframe parts has been developed by Fraunhofer in the Eco-Design Transversal Activity (ECO-TA) Platform of Clean Sky. Clean Sky, a Public Private Partnership between the European Commission and the Aeronautical Industry and the largest European aeronautics research programme, funds research activities to deliver significant reduction of the environmental footprint of aviation. In order to ensure the environmental improvement of the new materials and technologies, Fraunhofer performs the Life Cycle Assessment (LCA) of some airframe demonstrators, such as thermoset wing, thermoplastic and metallic fuselage, and cabin interior parts. However, one of the main challenges is the missing LCI data. Since the database is the core of an LCA, Fraunhofer itself has developed life cycle inventories of various chemical products used in the manufacturing of metal and polymer parts and in recycling processes related to airplane applications. Moreover, in Clean Sky the new technologies have to be developed in accordance with REACH - Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals. These technologies make use of chemicals such as corrosion inhibiting primer based on leaching inhibitor technology (Strontium chloride, Zinc phosphate); chemical solvent stripping (paint removal non-phenolic) and preparation of sol-gel solutions (3-(Trimethoxysilyl)propyl glycidyl ether, Trichlorsilan), which are not available in the current LCA databases (open source data, OpenLCA, ecoinvent, GaBi, etc). As one of Fraunhofer's institutes specialised in chemistry, Fraunhofer ICT (Institute for Chemical Technology) has the necessary know-how and infrastructure to gather more accurate data and develop new and more reliable LCIs for chemical products. At the moment, more than 60 new LCIs are being modelled by Fraunhofer ICT, with the development of a new classification system that aims at creating different clusters of chemical traits, processes and uses that will improve usability and accessibility of said data. With the development of these LCIs and database, the classification and the environmental impact – especially the toxicity – of these chemicals can be better assessed and compared.

5.06.P-Tu298 Filling ProScale Hazard Factor Data Gaps for Unclassified Substances

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The ProScale method is one of the tools in the Mistra SafeChem SSbD toolbox and calculates the toxicological potential for substances and mixtures with an exposure/dose factor and a hazard factor (HF) to be used for characterization of chemicals in life cycle assessments and chemical footprints. ProScale Hazard Factors (HF) are based on data from classification of chemicals in GHS and CLP. For small volume chemicals and new chemicals, data are scarce. By obtaining data typically used for classification purposes, a relevant interim HF could be derived. Relatively much data is needed so as a first step for an initial ProScale screening a simplified approach is suggested, making use of the precautionary principle. In ProScale, the highest possible HF has a value of 100 000. So this value could be assigned by the practitioner to all unknown substances in the first iteration of assessment of a system. Only if these substances are contributing significantly to the overall impact, there will be a need to look for more data and refine the HF.

5.06.P-Tu299 Gaps in Chemical Sector Life Cycle Inventories of Main Inventory Databases and How to Fill Them

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The life cycle inventories (LCIs) of chemicals are central to life cycle assessment (LCA) studies for a broad range of products, for example in the agriculture, healthcare, food, plastics or construction sector. Thus, maintaining high data quality is vital, while the complexity of chemical supply chains and the high diversity of products and processes makes this challenging. From a review of the main unit-process LCI databases (ecoinvent, IDEA, USLCI), we identify the gaps in chemical sector LCI data. The two most critical gaps are in the energy inputs, both for heat and electricity, and in the coverage of new process routes deployed in recent years. In terms of energy inputs, we observe that energy supply is frequently modeled with generic datasets such as national or regional electricity supply mixes, as well as generic steam supply. In contrast, we find about 7'000 power plants owned and operated by chemical companies, of which most are fossil fuel-based. We calculate the emission intensities of these power plants with a detailed unit-level model and utilize them to quantify environmental impacts of electricity and heat supply specifically for chemicals production. In addition, we model several key technologies for chemical production, that have gained major market shares in recent years and have not been covered by the main LCI databases, yet. With the results, we are then able to show at

high spatial resolution how the environmental impacts of chemicals production differ due to specific energy inputs, and which new technologies perform environmentally very differently from established technologies covered by the main life cycle inventories. Central roles for portraying the environmental impacts of today's chemicals production have the disproportionately large share of fossil fuel use in heat supply, and the massive expansion of coal-based chemicals production in Asia. In consequence, the results can be used to prioritize chemical sector updates and data improvements for main LCI databases.

5.06.P-Tu300 Development of a Site-Specific LCA Model to Assess Pathways Towards a Net-Zero Chemical Industry

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The chemical industry is responsible for 18% of the global industrial greenhouse gas emissions. To reduce this impact, various alternative technologies for reducing greenhouse gas emissions are being discussed for the chemical industry. However, environmental assessments to a net-zero chemical and plastic industry are available only on a global scale. Thus, the required changes in the chemical production sites on a more regional level to achieve net-zero emission chemicals are currently unknown. Precisely regional data as the demand for future raw materials are important for policy decisions in a country to plan infrastructure development, investment in new plants, and import strategies. To fill this gap, we will present a new chemical production site-specific, life cycle assessment (LCA) based model that can be used to determine technology choices in chemical production sites while systematically considering the site-specific utility supply, the site-specific feedstock availability, and the trade between the technologies in a chemical production site and regions of the world. In a subsequent case study, the bottom-up LCA model is used to assess pathways towards a net-zero German chemical industry. The case study includes 592 chemical production plants in 54 production sites to produce 85 chemical products. The alternative technologies, electrification, water electrolysis, Carbon Capture and Utilization, and biomass utilization, are investigated for Germany. The assessment of the pathways shows that greenhouse gas emissions of chemical production can reach zero if a combination of different alternative technologies and electricity with a carbon footprint below 50g CO₂-eq per kWh is available. Furthermore, the pathways' assessment reveals site-level demand shifts of intermediate chemicals, resulting in a mass increase by a factor of 2.6. This case study shows that the developed chemical production site-specific LCA model can map complex production systems and provide regional results of a holistic life cycle assessment with a high level of detail. The results can be used to guide policy strategies towards a sustainable chemical and plastic industry.

5.07 Standardization and harmonization of life cycle assessment

5.07.T-01 Inconsistencies Regarding Product System Definition and Multifunctionality Solutions in ISO 14040-14044 & Proposed Amendments

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We focus on two aspects of the ISO 14040-14044 standards where inconsistencies are unfortunately persistent. We explain and argue these inconsistencies and propose possible amendments. The first issue relates with the specification of a product system. In LCA, the environmental impact of a product system is assessed. In the main text of the ISO 14040 standard, a product system is a "collection of unit processes with elementary and product flows, performing one or more defined functions, and which models the life cycle of a product". However, in the annex A of ISO 14040 is written that: "the products and processes studied in an LCA are those affected by the decision that the LCA intends to support". These statements are contradictory, as a product system cannot be both a consequence of a decision and a product life cycle. The first definition, i.e. considering a product life cycle, from the main text, is at the core of attributional LCA (ALCA). The other approach, concerning consequences of a decision as stated in annex A, aligns with the concept of consequential LCA (CLCA). The second issue deals about the solutions for multifunctionality. The multifunctionality issue concerns processes/systems providing multiple products that have different functions, and the assignment of impact among them. The ISO standards present a fixed hierarchy of a limited set of solutions. This procedure is contradictory with other text in the standard which states that the first step of the LCA method allows to specify a goal and scope, on which to base further methodological choices. For example, for ALCA and CLCA, only certain solutions for multifunctionality should be applied. To address the inconsistencies, we present two adapted overarching aims for the standards, for which amendments can be specified. The first is to consider a more open & general LCA standard, with the addition of a novel product system definition and a general approach for multifunctionality solutions. The second is to limit the standard to a certain LCA type, e.g. ALCA, and develop new standards for others. Finally, these inconsistencies are persistent, should not be ignored and addressed swiftly. It is up to the standard committee to select a certain proposed amendment or perhaps conceive others. In the meantime, we recommend for now to consider a more open & general LCA framework, which may be the best option in the long run.

5.07.T-02 System Expansion in ISO Documents

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The international standard on life cycle assessment (LCA) stipulates that, wherever possible, allocation should be avoided by subdividing the unit process or by expanding the product system to include the additional functions related to the coproducts. System expansion has been interpreted in different ways: to assess the full multifunctional system, or too create a monofunctional model by giving the system a credit for the avoided production of products substituted by the coproducts. Standardisation is a consensus process, and the outcome will depend on who is attending the meetings of the International Standardisation

organisation (ISO). The content of a standard can shift simply because the composition of the group developing the standard has changed. Thus, the annex explaining system expansion as substitution in ISO 14041 was removed when this standard was revised and integrated into ISO 14044, but a similar annex was added 14 years later in the most recent amendment of this standard. Meanwhile, the same subcommittee produced a technical report (ISO/TR 14049) that interprets system expansion as expansion into a multifunctional system. Perhaps more importantly, and more difficult to amend, various LCA-related standards are developed within different ISO committees, creates inconsistencies between the documents. For example, the standard on life cycle inventory analysis of steel products stipulates system expansion and interprets it to imply substitution. The standard for Environmental Product Declarations (EPDs) in the construction sector states that system expansion with substitution fits in consequential LCA but should not be used in attributional EPDs. The draft standard on LCA of biobased plastics explicitly excludes substitution from the interpretation of system expansion. The original version of ISO 14044 allowed for different interpretations of system expansion. The new annex makes it more difficult to reconcile this standard with other LCA-related documents from ISO. The annex also misleadingly indicates that the two interpretations of system expansion are mathematically the same. A consolation can be found in the fact that the annex is informative and not normative. This seems to allow for other interpretations of system expansion in ISO than the one presented in the annex.

5.07.T-03 Investigating the Link Between LIFE Cycle Assessment and Circularity Indicators: A SETAC/ACLCA Working Group

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Current research has indicated that circularity practices and circular economy (CE) methods do not always reduce environmental impacts. The aim of this research is to investigate the adoption of life cycle assessment (LCA) methods to improve the environmental impacts of circularity practices. As part of the SETAC forum, an interest group (IG) on Circularity & LCA was formed in partnership with the American Center for Life Cycle Assessment (ACLCA) to tackle related methodological and technical issues. The membership structure is intended to be a good balance between academia, industry and government/environmental agencies. The IG's research approach is summarized in four key steps: Defining Goals & Objectives, Literature Review & Gap Analysis, Ideation, and Experimentation. To meet the mission of the SETAC/ACLCA IG Circularity & LCA, we have organized into four sub-working groups (Sub-WGs) so that complementary tasks can be completed concurrently in an effective manner. In Sub-WG 1 a literature review is developed in order to clarify the existing circularity and LCA end-of-life indicators. In addition the current status of the implementation of CE indicators by companies is assessed. Sub-WG 2 investigates the existing approaches to evaluate the environmental impact of circularity through LCA. In a survey and expert discussion a list of "pain-points" and methodological gaps is collected and analyzed. Sub-WG 3 delivers a mapping of the trade-offs between environmental benefits and circularity performance throughout the product life cycle and its resource loops. In Sub-WG 4 use cases based on the outcomes of the other Sub-WGs are developed. The main goal is to identify successes, challenges and opportunities when applying CE models and standards. In conclusion, LCA methods and software do not provide clear guidance and approaches to including CE pathways, measuring impacts, and defining system boundaries. There are an increasing number of circularity indicators and lack of clear guidance on how to assess the CE. The indicators do not always provide insight on the environmental savings or benefits of implementing CE principles. In this context, an ISO Technical Committee (ISO/TC 323) on CE has been established to develop standards on general implementation approaches and metrics for CE. A standardized CE evaluation method should support strategic decisions and monitor the progress towards sustainable development. In future work, a guiding flowchart or logigram could help users to navigate between these indicators, as well as to aggregate existing approaches into an integrated solution.

5.07.T-04 Where Are All the Additives? Identifying Trends in Plastic Additive Inclusion in LCAs of Mechanical Recycling

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Pathways for circular plastics are key topics in today's Life Cycle Assessment (LCA) community. To ensure the accuracy and reliability of these studies, LCAs on circular plastic materials and circular plastic recycling scenarios must consider the impacts of the entire chemical composition of the plastic assessed. Plastic additives are essential to the production and reprocessing of all plastics (Hahladakis et al., 2018). Added throughout all stages of production and recycling, plastic additives make up between .01-70% of the net weight of plastic materials (Aurisano, et al., 2021; ECHA, 2020). However, recent studies into the flows and reporting of additives throughout the EU found between 6,000-10,000+ additives were underreported despite their common use in plastic materials (Aurisano et al., 2021; Wiesinger et al., 2021). When considering scenarios for circular plastic pathways in LCA, it is vital that the flows and impacts of plastic additives are accounted for in the discussion and life cycle inventory (LCI) of the study. Previous literature reviews of LCAs of plastics have speculated that additive exclusion is due to knowledge gaps or cutoff approaches but have not explored these suspicions in detail (Bishop et al., 2021; van Oers et al., 2012). This systematic literature review separates author talk about additives from additive data presence in the LCI to identify if additive exclusion, or inclusion, occurs due to knowledge gaps or data quality. Focusing on LCAs that assessed plastic circularity in mechanical recycling pathways, we identified 103 LCAs published in peer-reviewed journals from 2010-2020. Despite one-third of the authors discussing additives in the text and one fifth mentioning the importance of additives, we could not classify additive inclusion in

the data for one-half of all articles despite thorough reviews of the article text and all accompanying supplemental materials. This review highlights the importance of data transparency in published LCAs and underscores the difficulty in informing decision making due to the state of standardization and harmonization in LCA today.

5.07 Standardization and harmonization of life cycle assessment (Virtual Only)

5.07.V-01 A Streamlined Methodology to Integrate Life Cycle Thinking With Existing Environmental Sustainability Assessment Tools in Construction Sector

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The use of the multi-dimensional life cycle thinking approach can support quantitative and qualitative analysis of policies and help to assess the environmental sustainability of strategies, revealing gaps, challenges, and interactions. However, LCT studies seem too expensive for an extensive implementation. Moreover, findings are always complex and a more transparent communication to non expert stakeholders is needed. In contrast, other environmental assessment tools are less robust, but more easily applicable, comprehensible, and communicable. As emerged from existing research, different assessment tools of policies give different environmental results, both in literature and in case studies. Therefore, the use of a single indicator seems not appropriate to define clearly if a policy is more sustainable than another and to avoid the risk of greenwashing. The present research suggests a step-by-step methodology to verify advantages when the quantitative results of a comprehensive LCT approach are combined with qualitative outcomes of other tools. Phases considered in the method are anticipation and problem definition, impact assessment based on quantitative research, policy implementation, and strategies evaluation. The step-by-step methodology is revealed helpful to plan analysis and to conduct data collection and interpretation. As a case study, the Environmental Product Declaration of an “eco-concrete” is performed and compared to qualitative analysis based on Italian legislation on constructions works and other existing environmental labels. Results show that environmental product declaration highlights some areas of interest (efficiency, comprehensiveness, space boundaries) but non hugely consider other aspects (i.e. trade off, and social or economic issues). Moreover, existing approaches seem focus mainly on some stages of the life cycle of concrete, disregarding others (end of life, product use, facility buildings, transport conditions). Rebound effects and degrowth patterns are also investigated. The step-by-step method proposed appears useful to include in local policies for the construction sector both environmental impacts and economic and social aspects of sustainability. The approach is revealed flexible and usable for products, services, strategies at various territorial level. Therefore, future uses of the method for other policies are wished.

5.07.V-02 Environmental Assessment of an Offshore Wind Farm With Concrete-Based Floating Substructures to Support 15MW Turbines

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Ever since the onset of transition from onshore to offshore wind power, there has been a constant effort to move the turbines further from the coast to the deep-water areas, which requires floating substructures. One innovative alternative for offshore wind floating substructures is based on concrete materials. The environmental impact of this new concept needs to be assessed through the resources demand along as its life cycle. COREWIND is a European project that aims to the reduce costs of floating substructures designed to carry wind turbines of 15MW in deep offshore marine environments. In this work, a prospective fully comprehensive life cycle assessment (LCA) of monolithic concrete spar platform for 15MW offshore turbines located in Gran Canaria Coast-Spain, has been performed through the production, installation, operation and maintenance, decommissioning and End-of-Life (EoL) recycling stages. The production stage, including the transportation of raw materials to the manufacturing facilities, shows the highest contribution in all environmental impact categories studied, while the impacts associated with the EoL stage bring benefits due to the material recycling considered in the model, whose contribution ranges from -11% to -42% through all impact categories. When focusing on the global warming impact category, the overall result is 7.23 gCO₂ eq/kWh, being below average from studies found in the literature (19 gCO₂ eq/kWh), which entails carbon payback times between 0.5 and 0.9 years considering future emission factors of the electricity grid mix in the European Union. The floating structure manufacturing entails 1.45 gCO₂ eq/kWh (24% of contribution in the manufacturing stage), which positions the use of concrete support structures for offshore wind to power the renewable energy transition.

5.07.V-03 Towards the Operationalisation of Life Cycle Sustainability Assessment: Integrating User Needs in LCSA Methodology Development

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The assessment of sustainability impacts of products is more and more requested by EU customers, investors, and regulators. Principles and requirements for a comprehensive sustainability assessment of products are described in the life cycle sustainability assessment (LCSA) framework proposed by UNEP/SETAC in 2011, according to which LCSA is the combination of environmental life cycle assessment (LCA), social life cycle assessment (SLCA) and life cycle costing (LCC) approaches. However, conducting an LCSA requires interdisciplinary knowledge and access to modelling tools and information that may not always be directly available and accessible. This means the existence of some entry-level barriers, which hamper the operationalisation of LCSA. It is thus necessary to understand the main needs and requirements from potential users of LCSA, i.e., which characteristics LCSA should have for being applicable, useful and affordable. Users' needs and wishes on LCSA have been investigated in the framework of ORIENTING (Operational Life Cycle Sustainability Assessment Methodology Supporting

Decisions Towards a Circular Economy), a EU H2020 project aimed at developing a robust and operational methodology for the LCSA of products (goods and services). A workshop was organised in April 2021 to discuss needs and wishes of users together with around 150 stakeholders, including academia and research representatives, policy makers, civil society, financial institutions, and industry representatives (both SMEs and large corporations). Flexibility of scope, transparency of results and support for interpretation of the results were among the key aspects highlighted during the workshop. The reflection of these needs into the LCSA methodology is currently under development, further strengthened by i) the connection with the standardisation activities, and ii) the testing and validation of the methodology in practical case studies. Environmental assessment will be conducted according to the PEF method, applying the category rules documents (when available for the case products). One of the aims of the project is to consider, how the PEF could be extended towards a comprehensive Product Sustainability Footprint in future. Additional consultation processes will take place in 2022 to further refine the LCSA methodology, which will be delivered in its final status by the end of 2023.

5.07 Standardization and harmonization of life cycle assessment (Poster)

5.07.P-Th183 Recycling Approaches in Lca-Related International Standards

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Recycling of materials from one life cycle into another introduces an allocation problem in life cycle assessment (LCA). The problem can be defined as the task of partitioning the impacts of common processes between the life cycles where the material is used. Alternatively, it can be defined as the task to partition the environmental impacts and environmental benefits between the life cycle that generates material for recycling and the life cycle where the recycled material is used. Existing scientific literature and LCA guidelines recommend very different approaches on how to deal with this allocation problem. Even standards developed within the International Organization for Standardization (ISO) diverge in the methods suggested, particularly in cases when the recycled material has a lower quality compared to primary material. The international standard on LCA (ISO 14044) states that quality losses can be accounted for, but without stipulating a method. A technical report guiding the application of ISO 14044 (ISO/TR 14049) suggests that, in the case of quality losses, the impacts of primary-material production can be equally divided between all products in which the material is used. The standard for carbon footprint (ISO 14067) includes a method that partitions the impacts of primary-material production between the first and the last product where this material is lost. The standard for Environmental Product Declarations in the construction sector (ISO 21930) assigns the full impact of primary-material production to the product where the primary material is used, although the environmental benefit of recycling can be included as additional, in a voluntary Module D. When there is no loss of quality in the material, most LCA-related international standards suggest or stipulate that the net environmental benefit of recycling can or should be assigned to the product that generates material for recycling, when there is no loss of quality in the material. An exception is ISO 21930, which does not give any credit for recycling in the calculations, but only as additional information in Module D.

5.07.P-Th184 Sensitivity Analysis of the Use of Allocation Methods on Slaughterhouse Level: A Case Study on Danish Pork Production

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Life Cycle Assessment (LCA) is a method used to assess the environmental impacts of products throughout their entire life cycle. When applied in livestock systems, it faces the problem of multiple outputs. The meat supply chain, particularly from slaughterhouse gate, produces many products and byproducts with multiple usages, values and actors involved. Current literature suggests that the allocation method chosen to divide impacts among products and byproducts is an important issue, since it may change the conclusions about a product's environmental impact. Four different allocation methods emerged from a literature review: system expansion, biophysical, economic, and mass allocation. The allocation methods in the guidelines and scientific articles showed that several of them follow the ISO standards closely (avoid allocation if possible, if not, follow hierarchy of allocation rules). Thus, their precedences are no clearer than those of the ISO standards. The Product Category Rules for red meat recommends using economic allocation on slaughterhouse level. However, economic allocation is often criticized by meat processors for its sensitivity to prices fluctuations in the market. In the present study, an LCA on Danish pork production was performed in which the potential impacts resulting from use of different allocation methods on slaughterhouse level was compared, followed by a sensitivity analysis of price fluctuations within the economic allocation. The results from the study suggests that economic allocation is stable despite price fluctuations in the market because the shares in the total proceeds remain stable over time. Economic allocation reacts to changes within the production when increasing the relative yields for human consumption and let the economically main product bear the main environmental burden. Mass allocation, assesses the same impacts to the product and byproduct and thereby does not stimulate to increased utilization of the live animal. Biophysical allocation reflects the underlying mechanisms of building tissues upstream while disregarding their fates downstream. System expansion is reflecting the utilization of the byproducts from the slaughterhouse. The different allocation methods gave very different results for the pork product. Hence, to achieve comparability and reduce the possibility of masking and manipulating results by selecting more convenient allocation methods, the same method should be applied in comparable studies.

5.07.P-Th185 Recovering Treated Sewage Sludge Nutrients for Agricultural Use: Life Cycle Assessment for a Circular Economy

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Utilizing treated sewage sludge (SS) has been perceived as means to close the product-life-cycle loop in agriculture by embracing the cradle-to-cradle philosophy of circular economy transition (CE). Within CE context, management of wastewater treatment

sludge has undergone a paradigm shift of being considered as waste to burden societies to a value-added material that could use to produced energy or a source to recover essential elements. The aim in this study is to assess the lost opportunity associated with disposing treated SS as opposed to recirculating its valuable constituents to support CE. The SS nutrients are modeled as CE consumables to an 'open-loop' system that is also subject to end of life (EoL) allocation in LCA as indicated by JRC (2010) guidelines. Consequential modeling system paradigm is applied, to avoid coproduct allocation by system expansion. The functional unit is defined in terms of the amounts of nitrogen (N), phosphorus (P) and potassium (K) recirculated from the treated sewage sludge produced annually in Kuwait. The results indicate a reduction in environmental burden with respect to fossil fuel depletion, metal depletion and climate change. A total of 95% of the reduction is realized by avoiding virgin nitrogen production and instead using its recirculated counterpart. Considerable amounts of natural gas, coal, dinitrogen monoxide (nitrous oxide, N₂O) and copper are consumed during virgin N fertilizer production. Because P is an essential nutrient, that underpins global food security, we use LCA to assess using SS as secondary sources of P compared to that recovered using sewage sludge ash (SSA). The results indicated that P from phosphatic rock had almost three times the burden borne by the current treatment of sludge and 1.6 the burden of SSA. The current system had a slightly smaller environmental impact compared SSA, except for climate change due to extensive Methane and CO₂ production in the current system. SSA approach could be particularly relevant for countries, such as Kuwait where agricultural reuse of SS is not generally accepted by the general public. Treated SS needs to be tested for microbiological agents (viruses, bacteria, parasites and helminths), chemical substances and heavy metals to avoid risks to public and occupational health via direct or indirect exposure.

5.07.P-Th186 The Overlooked Nutrient Substitution Issue in LCA

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Limitation on natural resources and the pursuit of sustainable development have promoted material recycling from waste streams. One good example is to use municipal biowaste or agricultural residuals in biological treatment and to reuse the digestate or compost as soil conditioners and fertilizers. Life cycle assessment has been widely applied in the nutrient recycling field to identify potential environmental consequences and trade-offs. Due to the nature of the recycling process, dealing with multifunctionality is then an inevitable and crucial question. Substitution is one of the common approaches to solve multifunctionality. It keeps mass balances intact and is in line with LCA that supports decision support. Previous studies have highlighted the inconsistency in substitution and the possibility of over-/underestimation due to different substitution approaches that are applied. However, the specialty of soil nutrient substitution has hardly been captured and discussed as a separate methodological topic. Furthermore, no guidelines and frameworks address nutrient substitution specifically. The LCA community has acknowledged the importance of the substitution approach but overlooked the specific demand from explicit sectors. This blind spot has obstructed the comparability, accuracy, and comprehensiveness of LCA. My proposed presentation will offer insights into the inconsistency and the potential methodological shortcomings of soil nutrient substitution. With the result of an undergoing systematic literature review, limitations and further directions regarding this issue will be shared. The results shall contribute to the development of a structured nutrient substitution methodology and promote sector-specific guidelines.

5.07.P-Th187 Review of LCAs Based on Chemical Processes

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Chemical production is essential for helping society to achieve sustainable progress. Green chemistry innovative reactions can contribute to achieving some Sustainable Development Goals (SDGs), as reported by the Global Chemicals Outlook-II (GCO-II). In particular: Zero hunger (SDG 2), Human health and well-being (SDG 3), Clean water and sanitation (SDG 6), Affordable and clean energy (SDG 7), Responsible consumption (SDG 12) and Climate change (SDG 13). The chemical sector is currently a \$4 trillion global business, employing more than 20 million people directly and indirectly and affecting more than 95% of manufactured products. The holistic approach of Life Cycle Analysis (LCA) applied to the design and evaluation of chemical products and processes has aided scientists in looking into the environmental impacts of their life cycle, comprising the raw material procurement, synthesis, use and end-of-life. The proposed presentation aims to review and compare publications of 47 industrial-scale chemical processes LCAs from 2012 to 2021 indexed in three of the most common scientific databases: Science Direct, Scopus, and Web of Science.

5.07.P-Th189 Environmental Footprint of Seafood Products Based on the Nexus Water-Energy-Food Approach

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During the last decades, the stakeholders within the seafood supply chain are raising the awareness of the environmental impacts related to fishing, aquaculture, processing, transportation, packaging and to all steps from farm to fork, increasing the demand of sustainable seafood products. Hence, there is a growing interest in calculating the environmental footprint of these products. However, there is not yet any standard methodology for calculating it. In the framework of the Interreg Atlantic area Neptunus project, it was developed a standardized framework to perform environmental footprint of seafood products based on life cycle assessment (LCA) perspective. The proposed framework delves into the typical issues when performing LCA such as the functional unit selection, system boundaries definition, inventory data modelling and identification of proper life cycle impact assessment methods. In addition, the Neptunus framework aims at the integration of environmental and nutritional indicators based on the NEXUS Energy-Food-Environment approach, allowing a better communication to the public. As a result of the integration, it was developed a single indicator to report not only the environmental performance of seafood products, but also nutritional issues related to the product on scope: the Nexus Water-Energy-Food. The methodology for the Nexus Water-Energy-

Food calculation comprises several stages: i) selection of environmental footprints, which include water, carbon, and energy footprints; ii) calculation; iii) normalization; iv) weighting; and v) communication, which includes the results communication throughout the designed NEXUS ecolabel. The application of the proposed framework to seafood products showed interested results in terms of impact based on fishing species, fishing ground, fishing gear, manufacturing processes, or ingredients added.

5.07.P-Th190 An Innovative Methodological Framework to Perform Life Cycle Assessment of Tourist Activities

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As whatever human activity, tourism is not exempt from environmental impacts related to the stay in destination. Hence, tourism implies travelling (road, air, and sea), accommodation, catering, and activities (social events, cultural visits, adventure, etc.). Traditionally, sustainability issues in tourism sector were addressed in terms of greenhouse gas (GHG) emissions, focusing on transportation from origin up to destination, disregarding accommodation, catering, and activities. Furthermore, up to now, life cycle assessment (LCA) of tourism activity has not been properly addressed due to the following issues: i) poor and limited life cycle inventory data; ii) reluctance of the sector; and iii) lack of a sectorial methodological framework. The Interreg SUDOE project Greentour — Circular economy and sustainable tourism in destinations of the SUDOE space, delves into the abovementioned issues developing and innovative methodology based on life cycle perspective. In this sense, the proposed approach includes the tourism activity as whole —i.e., from travelling, accommodation, drinking and eating, to the activities carried out in destination. In addition, the project proposes a methodological framework to deal with matters such as life cycle inventory modelling, allocation, system limits and exclusions, end of life, and life cycle impact assessment (LCIA) reporting. The framework developed is based on the requirements and provisions of other life cycle methodological frameworks such as ISO 14040, ISO 14046, PEF, ILCD, and PAS 2050 among others. Although the project is focused in SUDOE area and the pilot destinations Rías Baixas, Camino Lebaniego and Lloret de Mar (Spain), Guimaraes (Portugal), Ordino (Andorra), and Auvergne (France); the methodology can be used in other destinations, intending to become the reference framework for LCA in the field of tourism. As the LCIA results shall be reported to citizens, working groups in the field of sustainable tourism and public authorities, the environmental reporting (i.e., in terms of life cycle impact categories) was adapted to break the communication barriers when target audience is not familiar with LCA indicators. To do so, the impact pathways were evaluated from a life cycle impact results and the Sustainable Development Goals (SDGs) of the United Nations. As a result, the LCIA results were linked to the SDGs indicators related to tourism activities.

5.07.P-Th191 Towards a Sustainable Religious Tourism Under a Life Cycle Thinking: The CASE of Study of the Camino Lebaniego in Cantabria

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Tourism is one of the most important economic sectors in the world, contributing significantly to global gross domestic product (GDP). This contribution was forecast, before COVID-19, to grow at an annual 4%, increasing as consequence the greenhouse gas (GHG) emissions related to this activity. There many types of tourism activities based on the geographical and tourist demand, destination of travel, mode of transportation or means of accommodation. In particular, religious tourism is gaining special attention in many countries, such as the pilgrimage in Spain. In this sense, the main objective of this research is to develop an integrated sustainable model of the pilgrimage to the *Camino Lebaniego* in the region of Cantabria (Spain), which is one of the most popular routes in northern Spain. This study applies the methodology of Life Cycle Assessment (LCA) to evaluate the environmental impact and hotspots of the pilgrimage in this region. For this purpose, the selected functional unit (FU) is “*a pilgrim walking the route in three days*” and the sub-sectors of accommodation, food and beverage and waste management are included in the system boundaries. The results show that the pilgrimage of the *Camino Lebaniego* generates a total global warming impact of 12.30 kg CO₂ eq./FU, where accommodation contributes almost 66%, food and drink 26%, and waste management is almost negligible, with 8%. Electricity consumption in the hostel and the dinner contribute almost 85% of the total impact. In order to reduce the high impact of electricity consumption, alternatives are proposed such as the use of other types of heating such as natural gas or pellet boilers instead of electric heating. Likewise, if a meat-based diet is considered (13.65 kg CO₂ eq./FU) the impact is significantly higher than if a vegetarian diet is consumed (2.63 kg CO₂ eq./FU). Finally, origin of the pilgrim and the type of transportation were analysed, finding a higher impact as the distance increased. For instance, the impact of a pilgrim from Cantabria going by car is 6.55 kg CO₂ eq./pilgrim, compared to 171.46 kg CO₂ eq./pilgrim of German pilgrim coming by plane. Finally, these aspects are discussed and improvement measures are proposed to reduce GHG emissions through the introduction of good practices and environmental commitments with the pilgrims themselves.

5.07.P-Th192 Towards Harmonized Methodology to Indicate Neutralization Potential of Carbon Sequestered in Perennial Crop Systems

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To achieve climate targets, **long-standing removal of atmospheric CO₂ through land management** is essential in addition to **deep reductions in greenhouse gas (GHG) emissions** from agriculture and other sectors. Most current methods and tools for product and corporate GHG accounting, however, exclude the influence of land-based GHG emissions and CO₂ removals in

relation to land management. In order to address this methodological gap and to avoid non-science based claims, **harmonized methods are needed**. This project aims to fill the gap in current GHG accounting related to carbon sequestration and emissions associated with perennial biomass by **developing a common public methodology**. A generic model has been parameterized for apple, citrus, cocoa and coffee, based on empirical data. The model evaluates crop biomass compartments (i.e. branches, roots, stem and leaves), which is key to estimating carbon sequestration and emissions. This methodology describes the underlying procedure to parameterize a generic model to estimate biomass for selected commercially relevant typologies, and the framework to use these estimations in GHG accounting. Specifically, for GHG accounting only additional carbon sequestration following a land management or practice change (i.e. the difference between the average stock of a new state and a reference state) can be accounted for, and future reversibility of carbon sequestration in perennial systems (e.g. due to planting cycles or unexpected events such as fires) also must be considered. The final outcome of this work is to provide the groundwork for a harmonized methodology to indicate the neutralization potential (-CO₂e) of the carbon sequestered in perennial cropping systems.

5.07.P-Th193 The PEFCR on Batteries: Applicable to Smartphone Batteries?

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Smartphones have a high market penetration raising the awareness of the environmental impacts of these products. Life Cycle Assessment (LCA) studies of smartphone batteries are scarce in the scientific literature. The environmental impacts of smartphones have already been studied, highlighting the impact of the manufacturing stage. However, only one study focused on its battery [1]. To ensure the comparability of LCA results, harmonization of methods is needed. In that sense, the Product Environmental Footprint Category Rules (PEFCR) for High Specific Energy Rechargeable Batteries for Mobile Applications provide a methodological framework for LCA of batteries, even though not intended for research purposes. These PEFCR are applicable for batteries for electric vehicles and Information & Communication Technologies such as smartphones [2]. However, these PEFCR are often not used in scientific literature, partly due to their recent publication. Besides this issue, the PEFCR are not related to the various goals LCA studies can have. This study questions the relevance of these PEFCR to a self-healing smartphone battery developed in the context of the BAT4EVER project. Thanks to the increased battery performance, the possible second life of the battery will also be studied. Issues regarding the goal and scope of the PEFCR will be discussed, such as the choice of the Functional Unit (FU). The FU is defined as “1 kWh of the total energy provided over the service life by the battery system” which represents the function of a battery but is not widely used in literature. This total energy is based on battery industry standards that have been withdrawn and are not representative of the use of a smartphone. The inventory and the data need matrix will be compared to the data LCA experts frequently have. Most LCA experts usually do not have access to test results for the battery lifetime, or cannot calculate the energy efficiency as described in the PEFCR. The PEFCR recycling process is a pyrometallurgical treatment, followed by hydrometallurgical treatment, but other processes exist (such as hydrometallurgy and direct recycling) and are not described. In addition, the second life of batteries will be discussed as it is still not present in the current version of the PEFCR. Besides these issues, the PEFCR do not mention interpretation or sensitivity analysis of the results, calling for harmonization in this stage. **Acknowledgements:** This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the grant agreement No. 957225. **References**[1] Mejame PPM, Jung DY, Lee H, Lee DS, Lim SR. Effect of technological developments for smartphone lithium battery on metal-derived resource depletion and toxicity potentials. *Resour Conserv Recycl* 2020;158:104797. doi:10.1016/J.RESCONREC.2020.104797. [2] Siret C, Tytgat J, Ebert T, Mistry M, Thirdlaway C, Schutz B, et al. PEFCR-Product Environmental Footprint Category Rules for High Specific Energy Rechargeable Batteries for Mobile Applications. 2020.

5.07.P-Th194 Life Cycle Assessments (LCA) of a Heterogenized Ruthenium Catalyst Molecular Anode for Water Oxidation Catalysis

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An artificial leaf is a concept which is an experimental design, to be coupled with a renewable energy source. There are very few impact studies on a water electrolysis system for hydrogen production, with an impact study on a ruthenium-based molecular anode not being in existence at the time of writing. Therefore, Life Cycle Assessment (LCA) was used to generate a compilation and evaluation of the inputs, outputs and potential environmental impacts of a ruthenium-based molecular anode. A preliminary cradle-to-gate study is carried out to understand the net primary energy requirement of each stage of manufacture and net energy gain as a function of efficiency and the longevity of the molecular anode. Due to the complexity of the synthesis for the molecular anode, which included all the steps for the synthesis of the complex, the polymer and the attachment onto the polymer, many steps for manufacture required modelling. Reported inventory data was often unavailable due to the molecules not being in widespread use or modelled previously in any previous study. It was often necessary to resort to patents and even real-world laboratory data from experiments. Through this methodology, we were able to identify different points in the manufacturing process where the environmental impact was most acute. This enabled quantification of how much impact the use of ruthenium had on the overall LCA. It also enabled identification of problematic steps in the synthesis of the equatorial ligand, particularly the use of a common reagent used as an electrophilic cyanide source and the accumulative energy demand required for heating at different points of manufacture. This modelling was a useful process further down the line, since it is novel due to it being the first preliminary modelling of a molecular anode for water oxidation, it also provided data which will be useful when coupling this molecular anode with all the other components parts of an artificial leaf.

Track 6: Environmental policy, risk management, and science communication

6.01 Advances in the Regulation and Life Cycle Assessment of Metals in the Environment

6.01.T-01 Assessing Potential Environmental Risks From the Use of Zinc-Based Medicines in Piglets

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Zinc oxide has been used as a medicine to prevent diarrhoea in piglets during weaning but concerns regarding potential risk to the environment from the resulting zinc in manures spread on agricultural land have led to restrictions on its use in Europe. Concerns about the scientific robustness of the evaluation that led to the restrictions in Europe, and its relevance to important pig farming regions in the UK, led the Veterinary Medicines Directorate to evaluate the potential risks to the environment from this specific use of zinc for several UK soil scenarios. The evaluation of potential risks to the environment used the Intermediate Dynamic Model for Metals to calculate the loadings of zinc in agricultural soils receiving manures, and the resulting levels of zinc in local surface waters and their sediments over several decades of use. The evaluation of the potential risks due to zinc took account of zinc bioavailability in the soils, surface waters, and sediments. This presentation outlines the approach followed to perform the risk evaluation for the environment.

6.01.T-02 Assessment of the Potential for the Use of Lead Ammunition at Shooting Ranges to Contaminate Groundwater and Drinking Water

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Extremely high concentrations of lead have been measured in near surface soils at shooting ranges in Europe, for example up to 30-40% Pb has been recorded in the drop zone of clay target shooting ranges. Certain soil conditions enhance the solubilisation and mobilisation of lead through the soil profile, and the toxicity of this metal has led to concerns about the potential contamination of groundwater that may be abstracted as drinking water. This assessment has been undertaken during the development of ECHA's proposed restriction on the use of lead in ammunition and has sought to determine which soil and hydrogeological conditions are likely to present the greatest risk of lead migration from shooting ranges to groundwater and the extent to which these conditions are prevalent across the EU. A review of lead soil chemistry indicated that there will be greatest solubilisation and migration in acidic and organic rich soils with coarse soil texture, low Fe, Mn and ortho P. The hydrogeological assessment found that the connectivity between near surface soil, the vadose zone and underlying groundwaters is dependent on a combination of local factors, such as soil texture, soil chemistry and organic matter content, soil structure (particularly the existence of preferential flow pathways), local geology, topography, climate, and the depth to the saturated zone. It is difficult to estimate the prevalence and extent of groundwater vulnerability to lead contamination at shooting ranges at European, national or even regional levels because many of the contributing factors are local and difficult to predict at wider geographical scales. Local factors will always influence potential risks more than generic considerations, but areas with high intrinsic vulnerability (especially macropores and fracture flow) are likely to occur in all EU member states, although to differing extents. Lead in soil waters in the mg.L⁻¹ range have been measured in near surface soils but concentrations have been observed to rapidly decrease with depth and to date only a few studies have measured elevated lead in groundwater, with these being in near surface groundwaters under acidic soils (in areas that would generally be considered as wetlands).

6.01.T-03 Updating the Chronic Biotic Ligand Model for Nickel in Europe

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Nickel was identified as a Priority Substance under the Water Framework Directive and an Environmental Quality Standard was set in 2013 that applies throughout Europe. The EQS was set as a "bioavailable nickel" concentration, and the assessment of compliance against it should take account of bioavailability. The EQS was based on a Species Sensitivity Distribution for nickel that is normalised to the local site-specific water chemistry conditions using the Biotic Ligand Model. The ecotoxicity database that is used as the basis for the SSD, and the underlying models that define the bioavailability normalisation within the BLM, is based on information that was available in 2010. Several developments in the scientific basis of the Ni EQS have occurred since 2010, including additional ecotoxicity data that provide increased diversity in represented species, and the applicability of the underlying bioavailability normalisation models to broader ranges of water chemistry conditions. This presentation covers the inclusion of these data into an updated version of the chronic BLM for nickel. The chronic nickel BLM has been revised to update the ecotoxicity dataset and to enable bioavailability normalisation models to be applied over a wider range of water chemistry conditions than was possible previously. The most sensitive region for nickel toxicity was identified as Austria, due to the predominance of alpine stream waters, which have moderately high pH, low DOC concentrations and low or moderate calcium concentrations.

6.01.T-04 An Eu-Wide Assessment of Potential Nickel Risks Under the Water Framework Directive

Iain A Wilson¹, Adam Peters², Graham Merrington¹, Elizabeth Middleton³ and Christian E. Schlekot³, (1)WCA Environment Limited, United Kingdom, (2)wca, United Kingdom, (3)NiPERA Inc, United States

Nickel is a priority substance under the Water Framework Directive (WFD) and has a bioavailable environmental quality standard (EQS_{bioavailable}) of 4 µg L⁻¹. A procedure for deselection of current priority substances has been proposed by the European Commission. A substance that is deselected does not present an EU-wide risk, and is not required to be monitored by all Member

State countries. Unfortunately this procedure undertakes a comparison of the measured dissolved concentrations of nickel to the EQS, without accounting for bioavailability. We have performed a similar assessment, with the exception of using a comprehensive dataset covering as much of the EU-27 as possible. In addition, the data were processed to account for samples with concentrations that were less than the limit of detection. Site average concentrations were calculated in accordance with European Commission procedure and a tiered assessment was performed to assess site and sample exceedances. After data processing, the Spatial, Temporal and Extent of the EQS exceedances (STE) score was calculated, again in accordance with the European Commission procedure for three temporal subsets of the obtained data; all data from 2006 onwards (the European Commission procedure), data from 2013 onwards (the date the current EQS was set) and data from 2018 onwards (an assessment of data available from the last three years). The processed data were also evaluated against the further European Commission deselection criteria, including temporal trends. This platform presentation provides the outcomes of the European Commissions deselection procedure including the calculation of the STE score, when conducted using appropriate datasets and accounting for bioavailability. Implications of the outcomes of this work will be discussed in relation to European-wide nickel risks.

6.01.T-05 Calculating Comparative Toxicity Potentials With Metal-Specific Approaches: Does It Make a Difference?

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Within the life-cycle assessment (LCA) arena, the UNEP-SETAC life-cycle initiative has proposed to compare the impact of elementary flows on freshwater ecotoxicity using characterization factors (CF) derived by the USEtox model. However, several short-comings in the USEtox approach regarding specific metal issues have been identified, such as the lack of consideration of metal bioavailability and metal cycling within the environmental compartment. To overcome these issues, the life-cycle assessment arena has proposed to use the Free Ion Activity Model (FIAM) for incorporating metal bioavailability. However, this proposed approach still represents a simplification of real-life exposure situations as it does not account for all possible processes that influence metal transport in the aquatic environment and metal bioavailability effects to aquatic organisms. More complex approaches to model metal bioavailability and metal partitioning (Biotic Ligand Model [BLM] and Tableau Input Coupled Kinetic Equilibrium Transport-Unit World Model [TICKET-UWM], respectively) are available. The purpose of the current study is to compare the methods currently proposed for the derivation of characterization factors for metals in LCA and to evaluate these against the scientifically accepted metal-specific methodologies using Cu and Ni as case-metals. In that perspective, the CF values were calculated for Cu, and Ni for 7 freshwater Archetypes. More specifically, FF values were calculated based on fate modelling with the TICKET-UWM, the BF was calculated with speciation modelling in WHAM VII and the EF was calculated with the BLM approaches currently integrated in European risk assessment frameworks under REACH for Cu and Ni, as well as using the FIAM approach (using WHAM VII-speciation calculations). The use of the FIAM approach clearly increased variation in the Cu EF between Archetypes compared to the BLM-approach. This can be attributed to the omission of the competition effects of cations at the sites of toxicological action for metals for the FIAM approach. Relatively large differences in Cu FF, EF and to a lesser degree for BF were observed with values for the same Archetypes reported in literature, which can be explained based on the use of a simplified fate model in the USEtox framework, differences in toxicity databases and in speciation modelling assumptions. However, the discrepancies in FF, BF and/or EF are largely negated at the level of CF. Therefore, the range of the CFs calculated in the present study (partly) overlaps with those reported in literature. For Ni, the calculated FF, BF and EF_{FIAM} in the present study were comparable with those reported in literature, while larger differences were observed for EF_{BLM}. Hence, CF_{FIAM} were relatively comparable with those reported in literature, while CF_{BLM} tended to deviate more from reported values. In conclusion, differences in FF, EF and BF were observed when calculated using more complex, but more realistic metal approaches compared with values reported in literature. However, the discrepancies in FF, BF and/or EF are largely negated at the level of CF.

6.01 Advances in the Regulation and Life Cycle Assessment of Metals in the Environment (Virtual Only)

6.01.V-01 A Regulatory Application of a 30-year Soil and Sediment Background Metals Dataset

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The remediation of Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) or Superfund sites is limited to clean-up to levels no lower than background conditions. Naturally occurring background metals in soil and sediments often complicate this process. In effort to support cleanup decisions and policy development regarding metals in soil the North Carolina Department of Environmental Quality Division of Waste Management (DWM) compiled a statewide set of naturally occurring metals background data from publicly available reports of United States Environmental Protection Agency (EPA) Superfund site investigations. The dataset represents background conditions at 326 Superfund sites in the state of North Carolina, USA. It includes site location data and analytical measurements of 18 metals (Al, As, Ba, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Na, Ni, K, V, Zn, Hg) in 624 soil and 228 sediment samples representing site evaluations from 1985 to 2015. This 30-year dataset combines metals measurements acquired using the EPA Methods appropriate at the time of their analysis. To combine this diverse dataset, the presence of extreme outliers for all metals, and the need to perform a common analysis on all metals were both used to determine the nonparametric methods were used to describe the data. The data are presented in an interactive dashboard that presents summary statistics and graphical representations of the data. The dashboard includes user options for selecting and viewing the data in various ways so the presented information can be tailored to support specific types of decisions. The data and dashboard serve two main goals: 1) to support cleanup decisions and policy development regarding soil metals, such as

remediating parties' design of site-specific studies to define cleanup goals at contaminated sites, or North Carolina DWM's development of policies for managing off-site transfer of soils; and 2) to support public awareness of the levels of naturally occurring background metals in soil and sediments by providing communities the ability to investigate their own backyard and better understand background environmental conditions and understand anthropogenic contamination in a greater context.

6.01.V-02 Development and Validation of Multiple Linear Regression Models for Predicting Chronic Zinc Toxicity to Freshwater Algae in Australian Natural Waters

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Zinc is mined and refined extensively across Australia, so robust water quality guidelines are required to protect Australian freshwater ecosystems. To better manage and protect aquatic ecosystems, an improved understanding of how water chemistry influences zinc toxicity is required. Water chemistry parameters, such as dissolved organic carbon (DOC), pH and hardness are known to influence metal toxicity by modifying metal bioavailability. This study developed multiple linear regression (MLR) models to predict chronic zinc toxicity to a tropical freshwater alga *Chlorella sp.* based on EC10, EC20 and EC50 values from zinc-spiked laboratory waters. Developed models were validated using toxicity data from chronic toxicity tests using six natural waters covering a range of water chemistry parameters and geographies across Australia. Models were developed at the EC10, EC20 and EC50 level, both with and without interaction terms. In general, models performed better during auto-validation when interaction terms were included. At all effect levels, models with interaction terms predicted >80% of the observed data within a factor of 2 and >90% within a factor of 3. The MLRs consistently overpredicted zinc toxicity in all natural waters at all effect levels. Interestingly, the relationship between toxicity and the model parameters were generally consistent, with similar slopes in the data compared to the perfect agreement 1:1 line. This suggests that there may be a consistent factor not captured by pH, hardness, or DOC, causing this overprediction across the waters. Additionally, other MLRs developed using *Raphidocelis subcapitata* also provided poor and inconsistent predictions in the natural waters. The results of this study suggest that more toxicity modifying factors (other than pH, hardness, and DOC) may need to be considered when applying chronic algal bioavailability models to natural Australian waters. Further work is needed to better understand the effect of water chemistry on the mechanisms of zinc toxicity to *Chlorella sp.* which would allow for more appropriately developed algal bioavailability-based models and the subsequent use of these models in water quality guideline derivation for the Australian environment.

6.01.V-03 Regionalized Life Cycle Assessment of Global Sulfidic Copper Tailings: Dynamic, Site-Specific Approach

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Copper mining operations around the world have been dumping tailings as wastes on a massive scale. These residues, when poorly managed, carry environmental risks through metals leaching and long-term acid mine drainage. At present, the life cycle assessment of copper tailings is limited by data availability and model limitations. We provide a set of calculation approaches in our analysis to integrate state-of-the-art ore processing and geochemical models, evaluating the environmental impacts of tailings of 75% of the world's copper production (~400 individual sites). The methods comprise production data from market research and mineral processing handbook to construct site-specific life cycle inventories for tailings effluent. The leaching model involving geochemistry is employed to estimate releases of heavy metals from tailings at different time steps. Together with the ore composition and local hydrology, the change in pH value controls the leachability of tailings over time. Our results suggest that copper tailings from the large (i.e., porphyry) and medium-size copper deposits (i.e., volcanogenic massive sulfide and sediment-hosted) contribute to more than three-quarters of the total global freshwater ecotoxicity impacts of copper tailings. The former copper deposits are mined in the Americas (Chile, Peru, USA) and Asia (Indonesia, Papua New Guinea), while the latter are located in DR Congo, Zambia, Russia, and Poland. The hydrological condition strongly enhances metal leaching, especially over long-term horizons. The generated tailings inventories vary locally, even within single countries, indicating the importance of site-specific models and bottom-up data aggregation for country or global averages. Compared to averaged data in the previous database, our inventories show generally smaller impacts, but the estimated site-specific impacts can be higher for certain mine sites and countries. Our study provides regionalized inventories of copper tailings through the inclusion of a dynamic model, thus improving emission data precision for copper in life cycle assessments.

6.01.V-04 Towards Bioavailability-Based Guideline Values for Zinc in Australian and New Zealand Natural Waters

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Bioavailability models developed in the northern hemisphere cannot be adopted in Australia and New Zealand without validation due to (i) the high level of endemism found in local freshwaters, and (ii) distinct water chemistry characteristics, such as higher magnesium and lower dissolved organic carbon, which can influence metal bioavailability and toxicity. Toxicity modifying factors (TMFs) such as DOC and pH are only beginning to be incorporated into metal freshwater guidelines in Australia and New Zealand. This study investigated the applicability of existing chronic zinc bioavailability models to predict zinc toxicity in 12 uncontaminated natural waters (spiked with zinc) with varying pH, hardness and natural DOC. Model predicted toxicity was

compared to observed toxicity in chronic tests with sensitive local isolates of an alga *Chlorella* sp 12., the cladocerans *Ceriodaphnia cf dubia* and *Daphnia thomsoni*, as well as the green alga *Raphidocelis subcapitata*. A new chronic multiple linear regression model (MLR) was also developed for *Chlorella* sp. by varying pH, DOC and hardness in a laboratory water. Total and dissolved metals were determined in all toxicity tests, while ultrafiltered and DGT-labile zinc were also determined in all *Chlorella* toxicity tests. Existing Northern Hemisphere and NZ MLRs for *D. magna* based on EC50 values gave reasonable predictions of toxicity of zinc to *D. thomsoni* for a range of NZ waters. These EC50 MLR models could generally predict toxicity within a factor of two of the measured concentrations when the sensitivity coefficient was updated for *D. thomsoni*, which is more sensitive than the model species *D. magna*. Predictions were less accurate at the EC10 and EC20 level. TMFs had little influence on zinc toxicity to the Australian isolate of *C. dubia*, with a null model predicting EC20 values within a factor of two. The new MLR for *Chlorella* sp. consistently overpredicted zinc toxicity in the natural waters. Similarly, existing MLRs for *R. subcapitata* were also poor predictors of zinc toxicity to *Chlorella* sp., but were better for *R. subcapitata* in NZ waters. Further work is required to understand the effect of TMFs on mechanisms of zinc toxicity to microalgae before these bioavailability models can confidently be applied across algal species and to guideline value derivation in local waters.

6.01 Advances in the Regulation and Life Cycle Assessment of Metals in the Environment (Poster)

6.01.P-We243 Bio-Met a Simple Tool for Ni EQS Compliance Assessment

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Nickel is a Priority Substance under the Water Framework Directive and the EQS is therefore applied throughout the whole of Europe. Although the EQS was set in 2013 there are still some limitations in the way that Member States implement it. Whilst this issue is likely to be reduced somewhat by the recent publication of guidance on the implementation of bioavailability for metals it may also be limited to some extent by the usability of the simplified tools that are available to practitioners to apply bioavailability corrections. In many cases nickel is the only substance that Member States need to apply a bioavailability correction to that is based on a Biotic Ligand Model. The existing user-friendly tool bio-met has been modified to simplify its use for nickel bioavailability calculations and improve its usability whilst retaining the high level of accuracy provided by the model. This presentation outlines what changes have been made and how they help users in the regulatory community to properly perform the assessment of bioavailability for nickel in freshwaters.

6.01.P-We244 Nickel EQS in Europe: Implementation or Update

Adam Peters¹, Graham Merrington², Christian E. Schlekert³, Elizabeth Middleton³ and Emily Garman³, (1)wca, United Kingdom, (2)WCA Environment Limited, United Kingdom, (3)NiPERA Inc, United States

Nickel is a Priority Substance under the Water Framework Directive and the Environmental Quality Standard (EQS) is therefore applied throughout the whole of Europe. The EQS is set as a bioavailable nickel concentration, and therefore requires bioavailability to be taken into account to properly assess compliance against it. Technical guidance on the implementation of bioavailability-based standards for metals has recently been published by the European Commission. Implementation of the EQS for nickel has been very variable between different member states, and it is likely that this reflects the limited guidance that was available until recently. The EQS for nickel has been identified by the European Commission as potentially requiring an update, despite the limitations surrounding the proper implementation of the existing EQS. An update of the science that is used for the assessment of nickel bioavailability in European freshwaters suggests that the proportion of monitoring sites that would be likely to fail an updated EQS is very similar to the proportion of sites that would fail the existing EQS. This suggests that focusing on improving the implementation of the existing EQS for nickel may be a more appropriate use of the limited time and resources available, especially given the very recent publication of guidance on the implementation of bioavailability based EQS for metals.

6.01 Advances in the Regulation and Life Cycle Assessment of Metals in the Environment (Poster Corner)

6.01.PC-We07 Review of Metal Toxicity Testing With *Lymnaea stagnalis*

Adam Peters¹, Dean Leverett², Kate Roylance³, Graham Merrington⁴, Charlotte Nys⁵, Mohammed J. Chowdhury⁶, Adam C Ryan⁷, Stijn Baken⁸, William Stubblefield⁹, Christian E. Schlekert¹⁰, Elizabeth Middleton¹⁰, Emily Garman¹⁰, David Boyle¹¹, Christopher Cooper¹², Carrie Claytor¹³, Marie-Christine Simard¹⁴, Amiel Boullemant¹⁵ and Eric J. Van Genderen⁷, (1)wca, United Kingdom, (2)WCA-Environment Ltd, Faringdon, United Kingdom, (3)wca consulting, United Kingdom, (4)WCA Environment Limited, United Kingdom, (5)ARCHE Consulting, Belgium, (6)International Lead Association, United States, (7)International Zinc Association, United States, (8)European Copper Institute, Brussels, Belgium, (9)Oregon State University, United States, (10)NiPERA Inc, United States, (11)Cobalt Institute, Afghanistan, (12)International Zinc Association, Belgium, (13)Copper Development Association Inc., United States, (14)Rio Tinto, Afghanistan, (15)Rio Tinto Aluminium Pechiney, France

Although there is a standard OECD test guideline for chronic toxicity testing with adult *Lymnaea stagnalis* based on reproduction, most academic studies on this species have used juvenile growth tests. There is considerable variation in the methods and endpoints used in the studies, and very few follow the standard OECD reproduction test guideline. Several different food sources (e.g., lettuce, sweet potato, carrot, fish flakes) have been used for both maintaining the cultures and for the tests themselves that have been reviewed. Food source can have a considerable influence on the outcome of the tests, and this appears to be greatest for low effect levels on growth endpoints for juveniles. The nutritional quality of the food source used can have a considerable influence on the sensitivity of the test subjects to metal toxicity. There is considerable variation in both the methods and endpoints used for juvenile growth tests on *L. stagnalis*, and this severely limits any comparisons that can be made between the results of

different studies. For example, a significant number of tests have been conducted in waters with hardness levels considerably lower than those recommended by the OECD test guideline (140-250 mg/L as CaCO₃). Overall, relatively high levels of variability between replicates are not uncommon, and poor control performance including high mortality in controls has been observed in several cases. Control performance criteria based on the endpoints measured (i.e. growth) should be established for the juvenile growth test to enable the performance of different tests to be compared. The OECD test guideline should be adapted to include a standardised method for a juvenile growth test. A ring-test of any revised methods should be conducted to assess the variability of results between different testing laboratories.

6.01.PC-We09 Effects of Water Hardness on Zinc Toxicity in *Daphnia magna*

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Aquatic organisms are often exposed to a wide array of different heavy metals. Proper risk assessments are required to ensure safe environments. At present, most environmental risk assessments derive acceptable exposure levels based on measured levels of chemical pollutants with the addition of safety factors. Bioavailability models have been developed to predict metal toxicity as a result of variation in the local physicochemical conditions. However, due to the complexity of biological systems, the theoretically calculated set limit values represent non-optimal concentrations for essential trace metals such as zinc, due to having both a lower and upper limit for tolerance. While all living organisms require a minimum level of zinc for proper cell function, high concentrations can result in toxic effects. There is a growing need to connect water characteristics and bioavailability with biological endpoints such as mortality, reproduction, and gene expression. In this study, we analyzed the effects of different water hardness on zinc toxicity using the cladoceran *Daphnia magna*, a freshwater species widely used in ecological risk assessments. Exposure for 96 hours to increasing zinc concentrations in different water hardness significantly altered survival rates. Optimal zinc concentration for survival was observed to be inversely proportional for soft and medium water. However, increased hardness combined with low zinc concentrations led to higher mortality caused by zinc deficiency. Mortality rates were observed to be correlated with the ratio of the sum of the calcium and magnesium over zinc concentrations. Therefore, a competition-based model to describe the effects of hardness on zinc toxicity is proposed. The qPCR analysis was conducted to study different biological pathways involved in the metal response, stress response, oxidative response, and reproduction. Changing hardness resulted in distinct gene expression profiles at the same zinc concentrations. 21 days exposure was conducted to analyze reproduction success and development. Reproduction and development were altered by hardness and zinc. Our data indicate that hardness plays an important role in zinc toxicity by effectively changing the bioavailability at both physiological endpoints and gene expression. Thus, water characteristics and toxicogenomic analysis should be considered to improve environmental risk assessment.

6.01.PC-We10 Updating the Chronic BLM for Nickel in Europe

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Nickel is a Priority Substance under the Water Framework Directive and the Environmental Quality Standard (EQS) is therefore applied throughout the whole of Europe. The dataset upon which the EQS was based included research up to, and including, 2010. Since that time there have been developments in terms of both the ecotoxicity data that is available, and the bioavailability normalisation models that are used to calculate the site-specific nickel sensitivity of the ecosystem. The additional ecotoxicity data includes both additional data for species that were already represented in the database as well as information on additional species that are not represented in the database that is used for the existing EQS. The updated BLM includes 47 different species, 15 of which were not previously represented in the ecotoxicity database and can be applied over a broader range of water chemistry conditions than the model that is used for the existing Ni EQS. The predictions of the species-specific nickel bioavailability models have been validated laboratory tests on other species in natural waters that cover a wide range of the surface water conditions encountered in Europe and Australia, and the whole community predictions of the nickel BLM are protective of several mesocosm studies. An indicative compliance assessment shows that levels of compliance based on the updated Ni BLM would be very similar to those based on the existing EQS. The most sensitive region for nickel toxicity was identified as Austria, due to the predominance of alpine stream waters, which have moderately high pH, low DOC concentrations and low or moderate calcium concentrations.

6.01.PC-We11 Evaluation of the Priority Substance Deselection Assessment for Nickel

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Nickel is a Priority Substance under the Water Framework Directive, and the Environmental Quality Standard (EQS) is therefore applied throughout the whole of Europe. An approach for identifying substances that no longer pose a sufficiently widespread risk to surface waters has been developed by the European Commission and nickel has been included in their evaluation. There are several limitations to the analysis conducted by the European Commission, including assessing whether or not nickel exposures complied with the existing EQS several years before the EQS was actually derived or set, and failing to take account of bioavailability even though the EQS for nickel is a set as a “bioavailable nickel” concentration. This presentation summarises how the assessment of the appropriateness of deselecting nickel as a priority substance should be performed so that it is consistent with the existing EQS for nickel under the WFD. The presentation also draws conclusions based on a comprehensive evaluation that includes a proper consideration of nickel bioavailability in accordance with the nickel EQS.

6.02 Developments for regulatory risk assessment for birds and mammals under pesticide legislation

6.02.T-01 Revision of EFSA 'Risk Assessment for Birds and Mammals' Guidance Document

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In order to gain EU Level approval of a pesticide active substance or plant protection product under Regulation (EC) 1107/2009, it is necessary to demonstrate that there are no unacceptable risks to bird and wild mammals. In 2009, the European Food Safety Authority (EFSA) issued a guidance document ('Risk Assessment for Birds and Mammals', EFSA (2009)). EFSA was requested to update the guidance document clarifying certain aspects and accounting for the latest regulatory and scientific developments. A follow-up project has also been initiated to design an on-line calculator tool to implement the risk assessment methodology. In September of 2020 EFSA published a draft of the updated guidance and sought feedback from stakeholders and the public. This presentation will provide an overview of the tiered risk assessment approach for birds and mammals, an analysis of the comments received as part of the public consultation and discuss areas of the guidance which will be improved. The feedback and ideas for the calculator tool, which is being developed to facilitate the implementation of the revised risk assessment methodology, will be discussed. The current timelines for the revision will be given.

6.02.T-02 Revision of the EFSA Guidance Document on Birds and Mammals: Industry View

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The EFSA Guidance Document on the Risk Assessment for Birds and Mammals [EFSA, 2009] (EFSA GD) has been used for more than 12 years in the EU for the evaluation of Active Substances and Plant Protection Products. Recently, EFSA has launched the public consultation on the draft updated guidance document on Risk Assessment for Birds and Mammals. Industry will contribute by sharing the experience from risk assessors who have been using the EFSA GD on a daily basis, highlighting those areas where the update should put more emphasis on or requires further guidance. Contributing with specific expertise as for this occasion, industry have mobilized not only the Crop Life Europe Expert Group on Terrestrial Vertebrates but also the CLE Expert Groups on Statistics, Effect Modelling, Residue Definition, Toxicology and Efate. The risk assessment scheme of the current EFSA GD has proven generally adequate to evaluate the acute risks to birds and mammals while major issues have occurred with the refined risk assessments, particularly for the reproductive risk. Numerous changes have been proposed in the new draft EFSA GD while its impact cannot be evaluated as protection goals are still pending and a calculator tool has not been provided. In the opinion of risk assessors from industry, the main refinement parameters that require more discussion before their implementation in routine risk assessment in the future are the following: the Benchmark Dose approach and the selection of the ecotox relevant reproductive endpoint, the use of the f_{TWA} and agreed Tier 1 risk assessment, the selection of focal species of birds and mammals and further clarity or discussions on the higher tier options and modelling. These parameters will be described in more detail in the platform presentation. Targeted actions will contribute to minimize contradictory interpretations by risk assessors, increase the acceptance of, provide clarity and a path forward to a successful guidance.

6.02.T-03 The New Draft Guidance for Birds and Mammals From the Perspective of Practitioners

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The risk to wild birds and mammals from applications of Plant Protection Products (PPPs) is assessed in a tiered approach which is regulated in the EFSA Guidance Document (GD). Several refinement options are available. The highest tier and most realistic refinement option is to conduct generic field studies and field effect studies. However, the GD gives hardly any guidance for this kind of studies besides that they have to be adjusted to the focal species, the crop, the season, and the concerns raised by lower tier results. Despite the valuable contribution of field studies to the risk assessment of PPPs, results and their interpretation have been repeatedly questioned as well as their general value for the risk assessment procedure. The recently published draft of the updated EFSA GD for the risk assessment of birds and mammals aims to make a considerable improvement to strengthen the higher tier studies. The main challenge of higher tier studies remains to persuasively demonstrate their suitability and representability. Therefore, the draft GD requests additional information for higher tier studies, such as landscape characteristics, recent and previous-year use of PPPs and agricultural activities at the study sites and neighbouring fields of similar crop, and the food availability at the studied crop and off-crop areas. Here, we aim to pick up some points of the new GD on higher tier field studies and discuss them from the practitioner's point of view. Hence, if conducted appropriately, generic field studies and field effect studies offer the most realistic approach to study the risk from PPPs.

6.02.T-04 Revision of EFSA Birds and Mammal Guidance: Some Academic Reflections

Nico van den Brink, *Toxicology, Wageningen University, Wageningen, Netherlands*

Recently, the EFSA Birds and Mammal guidance has been revisited, not a complete overhaul, but an update. I'd like to

acknowledge the constraints of regulatory testing, especially with vertebrates. In some respects, the current guidance can be a balancing act, but given this, it is of high value. Here, I will highlight some scientific aspects that may need attention. The use of surrogate Protection Goals, especially with respect to the aspect of ‘no visible mortality’ may apply differently to species, according to their size and visibility, which should not be the base of their protection. Indirect effects on e.g. prey availability are currently not addressed, demanding for a clear harmonisation with other guidance’s on potential prey invertebrates or plants. Within the tiered approach of exposure assessment, impacts of some assumptions underlying the definitions of PT and PD on the uncertainty in the RA are not completely clear. These need further attention, as well as those underlying more complex landscape level monitoring. This is of importance with respect to the general approach of less conservative criteria at higher tiers where realism is deemed to be higher and uncertainty lower. The current guidance addresses oral exposures, it is recommendable to include dermal exposures of e.g. egg, chicks and juvenile wildlife that run the risk being over sprayed when in-crop. Finally, future revisions of both the guidance and the regulatory framework should include the operationalisation of new *in vitro-in vivo* tools and assays to minimise the use of test animals.

6.02 Developments for regulatory risk assessment for birds and mammals under pesticide legislation (Virtual Only)

6.02.V-01 Calibrating the EFSA Tier 1 Protection Level for Voles in the Long-Term Risk Assessment

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According to the draft update of the EFSA bird and mammal GD shared in autumn 2021 for public commenting, calibration of the protection level from the current and future Tier 1 scenarios with field data is recognized as a key research need. Under the current (2009) EFSA GD, common vole scenarios systematically fail the TER_{LT} of 5, but it has been questioned for a long time if this very resilient species requires the same level of protection as other mammalian species. In recognition of that high resilience, TER_{LT} thresholds for voles have already been lowered in a few EU countries. In years of mass occurrence, common voles become an agricultural pest and are the target of direct vole control methods such as rodenticide applications. However, most of the EU countries (and EFSA) still expect the standard TER_{LT} of 5 to be demonstrated in order to accept low risk. The TER_{LT} for voles can be refined to some extent by conducting plant residue decline studies, typically demonstrating foliage DT50 below the 10d default value. However, often there is the need to additionally employ population models (not yet well accepted) and/or field effect studies (technically challenging). Regulatory interpretation of such a field effect study is inevitably complex and difficult to predict. Thus, conducting and evaluating field studies to demonstrate safe use for each case on its own is lengthy and resource-demanding. Therefore, a project has been initiated to evaluate available field effect studies on common voles in order to determine whether the vole scenario requires the standard TER_{LT}, or whether a lower TER_{LT} could still provide a sufficient level of protection. This activity can be related to similar activities by EFSA and member states, e.g., the calibration of the bird TER in EFSA (2008) with field effect studies, the request from the Joint Working Group of Member State Risk Managers (2009) to “re-consider the need for the vole scenario, given the resilience of their populations”, or the concept of the “reference tier” in the Aquatic GD (2013). Over a decade has passed since the implementation of the EFSA Bird and Mammal GD 2009, subsequently a number of vole field effect studies has been conducted which would now allow such calibration exercise. This poster will present an overview on the status of the calibration project for the vole scenario, the analyses, the opportunities and difficulties encountered and the envisaged path forward.

6.02.V-02 Dissipation of Plant Protection Products on Freshly Drilled Seeds

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For seed treatments, the current long-term Tier 1 risk assessment for birds and mammals applies an Ftwa value of 1, assuming no dissipation of the active substance on the treated seeds. Partially due to this assumption, for most active substances applied as seed treatment the long-term Tier 1 risk assessment does not demonstrate an acceptable risk and further higher tier refinements are required. One possible refinement is to calculate a Ftwa value using active substance specific DT50 data based on seed residue trials, demonstrating an actual dissipation of the active substance on the treated seeds. This poster will present results from an evaluation of dissipation data from several industry-owned residue dissipation trials on treated seeds. Based on an appropriate kinetic evaluation a default Ftwa for the use in Tier 1 seed treatment risk assessments will be proposed.

6.02.V-03 Field Data Support Combining Refinements on Diet Composition (PD) and Proportion of Diet (PT) Obtained From Fields Newly Drilled With Treated Seeds: Results From an In-Field Enclosure Study With Wood Mice

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The EFSA Guidance Document for Risk Assessment to Birds and Mammals requires to calculate the chronic risk for birds and mammals exposed to seeds treated with plant protection products. Recently, a draft of an update of this guidance document was published. The updated document states without any scientific justification that a combined refinement of the diet composition obtained from the treated area (PD) and proportion of diet obtained from a treated area (PT) is not acceptable. By using the example of wood mice foraging in newly drilled cereal fields, it is shown that combining both PD and PT is valid and both factors should be regarded as independent refinements. When the recommendation in the updated GD is followed, wood mice are assumed to enter newly drilled fields and exclusively feed on contaminated seeds before emergence of the crop. However, wood

mice are known to be omnivorous, feeding on a mix of dietary items if present. To collect empirical evidence on in-field exposure, a field study was conducted. It was determined which food items are present in a newly drilled cereal field and which of these are consumed by wood mice. A 1 ha in-field enclosure was set up on a conventionally prepared newly drilled wheat field in central Germany. Wood mice were captured in the surrounding landscape and released into the enclosure directly after drilling. Seed density was artificially increased to mirror worst-case exposure conditions. Available dietary items and their abundance were determined. After two nights of foraging, the individual wood mice were re-captured, and their stomach contents examined to determine diet composition. Different dietary items were present inside the newly drilled cereal field. Also, as expected from an omnivorous species, wood mice fed on a mix of dietary items when confined to the in-field enclosure. In conclusion, a combined refinement of the diet compositions (PD) as well as the proportion of diet (PT) obtained in a treated area is justified since both parameters describe different behaviors of wood mice. Our findings thus contradict the recommendation laid out in the draft of the updated guidance document.

6.02.V-04 Laying Hen Studies and Time-To-Effect in Reproductive Risk Assessments for Birds

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For pesticides in the EU, the reproductive risk assessment for birds is usually based on 21-d TWA concentrations in feed items (EFSA GD 2009), at Tier 1 estimated with a default DT50 of 10 days. The draft revision of this guidance (2021) contains the view that no TWA be used for “effects on chick development” unless “significant evidence is provided to indicate that these effects are definitively linked to a long-term exposure”. There is no reasoning provided for this exceptionally strong wording, setting high hurdles for evidence in a situation where extra investigations with vertebrates are discouraged. There is also no reasoning why the standard assumption for effects on chick development would be “due to short-term exposure”, but it may be assumed that the concern is based on the short time frame of egg development before it is laid. The regulatory avian reproduction study cannot inform on the time course of exposure and effect because the birds in this study are already under exposure for 10 weeks before the first egg is developed. So the question is where this “significant evidence” may be found or generated. A possible starting point may be to ask how an observed effect on chick development (like impaired embryo growth in the egg) is actually induced. Recent method developments with egg injection of toxicants show that in-ovo exposure of embryos can generate such effects on chick development. Thus, the time course of residue transfer from the exposed hen into the egg may present the evidence for assessing the suitability of TWA concentrations. We present a case study for a fungicide inducing effects on chick weight in Bobwhite quails. Laying hen studies show that the residue in the eggs consists mainly of its metabolite. We conducted a study where the metabolite was injected into fertilised eggs, and assessed the growth of the embryos in the treated eggs one day prior to hatch. Results confirmed that in-ovo exposure to the metabolite reflects the effects on chick development seen in the reproduction study with the parent. The time course of residue concentrations in the laying hen egg residue study showed that an exposure of about 3 weeks is needed to reach plateau concentrations, suggesting that the 21-d TWA window may be a realistic setting for effects on chick development. This case study suggest that laying hen studies should be examined for possible evidence on the length of TWA-windows for effects on chick development in avian reproduction studies.

6.02 Developments for regulatory risk assessment for birds and mammals under pesticide legislation (Poster)

6.02.P-We245 Weed Seed Availability on Crop Fields - How to Make an Unknown Visible

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Food availability is a key factor for the attractiveness of bare soil fields as potential foraging habitat for birds and mammals. In accordance with the EFSA 2009 Guidance Document on pesticide risk assessment for birds and mammals and the 2021 draft and revised version the availability of weed seeds as wildlife food source on fields over-sprayed with pesticides has to be considered as exposure route. The study aimed to quantitatively identify the reduction of available weed seeds on the soil surface due to agricultural practice: harrowing and sowing in sugar beet fields. For this purpose, four types of weed seed surrogates (*Silybum marianum*, *Phacelia tanacetifolia*, *Trifolium repens*, *Sorghum bicolor*), dyed with fluorescent orange have been distributed on bare soil fields in Germany typically used for sugar beet cropping. Within study fields, four comparable study plots were established. The harrowing process, and the drilling of sugar beet seeds were conducted by local farmers shortly after the distribution of the weed seed surrogates. A black light box was used to determine the number of weed seeds visible after distribution on soil surface. The same procedure was conducted after harrowing and drilling of the sugar beet seeds to determine the impact of these agricultural processes on the number of weed seeds on soil surface before agricultural processes start in the spring drilling period. Results will be presented and discussed regarding the importance of this exposure route on bare soil fields after drilling and their potential use in the context of dietary risk assessment for granivorous and omnivorous birds and mammals.

6.02.P-We246 Deriving Ecorelevant Endpoints for Wild Mammals Under the Draft New Bird and Mammal Guidance (EFSA 2021)

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Under the EU regulation for authorising plant protection products (Regulation (EC) 1107/2009), the potential risks to wild mammals must be assessed. The current default approach for wild mammal risk assessments is to use the worst-case no observed adverse effect level (NOAEL) that was concluded from the toxicology package for the human health risk assessment. However, there are many parameters that are measured in toxicology studies that are relevant for individual humans but not for wild

mammal populations. The current bird and mammal guidance (EFSA 2009) theoretically allows the derivation of an ‘ecologically relevant’ wild mammal toxicity endpoint that only takes into account those effects relevant for wild mammal populations. It was later recommended by EFSA in 2015 that the assessment of ecological relevance should be made at the active substance level. In September 2021, EFSA released the draft new bird and mammal guidance, which includes recommendations for the derivation of ecorelevant endpoints for wild mammals. The aim of this presentation is to review the proposals under the draft new guidance (EFSA 2021) for deriving ecorelevant endpoints for wild mammals, including issues such as which studies to use, species sensitivity, types of effect, and magnitude of effect.

6.02.P-We247 Experimental Design and Statistical Analysis of Avian Reproductive Studies

John Green¹, Manousos Foudoulakis², Timothy Fredricks³, Thomas Bean⁴, Jonathan Maul⁵, Stephanie Plautz⁶, Pablo Valverde⁷, Adam Schapaugh³, Xiaoyi Sopko⁸ and Zhenglei Gao⁹, (1)JohnWGreen-ecostats.com, United States, (2)RAS, Corteva Agriscience, Peristeri, Greece, (3)Bayer Crop Science, United States, (4)FMC Agricultural Solutions, United States, (5)Syngenta Crop Protection Inc., United States, (6)BASF, United States, (7)Dow AgroSciences LLC, United States, (8)Corteva Agriscience™ The Agriculture Division of DowDuPont™, United States, (9)Bayer Crop Science, Germany

The draft update of the EFSA bird and mammal (B&M) GD shared in autumn 2021 for public commenting recommended the BMDL10 as the default relevant reproductive endpoint for both B&M. In addition, Historical Control Data (HCD) is currently not recommended for risk or hazard assessment without further guidance from toxicology experts. The Terrestrial Vertebrates ad hoc Team (TVahT) of the Crop Life Europe (CLE) Association discusses further the use of the BMD and has developed a statistical protocol for avian reproduction studies. This protocol includes guidelines for the use HCD in risk assessment and detailed statistical tools for estimating BMD10 with confidence bounds and determining a NOAEL when the data do not support BMD methodology, as well as guidance on which of these two approaches is justified. The judicious use of HCD improves risk assessment both by revealing treatment effects that are notably inside or outside the historical control range, and by identifying unusual concurrent controls that may obscure or exaggerate treatment effects. We recommend model averaging to incorporate model uncertainty more completely in risk and hazard assessment. In addition, improved model fit assessment reveals the effect of unusual observations and assures that model averaging is based on stable models not unduly influenced by outliers or poor fits. When BMD10 estimates are not supported by the data, we present methods to determine NOAELs that improve risk assessment that are also backed by case studies and computer simulations. Among the statistical tools are recommendations on the use of generalized linear and non-linear mixed models (GLMM and GNLMM) with extensive computer simulations and case studies that support these recommendations and contrast them to older methods. These methods are more consistent with the actual data collected than methods that require data transformations or force the data to fit a normal distribution. GLMM and GNLMM methods provide a consistent framework for the variety of response types encountered in avian reproduction studies. Also addressed is the experimental design for this type of study. It is shown to be possible to maintain the same total number of birds but allocate them differently to treatment groups and pens so as to increase the likelihood of quality BMD10 estimation without sacrificing the power of NOAEL determinations when that approach is needed.

6.02.P-We248 Focus Matters - Bird Focal Species for HIGHER Tier Risk Assessment

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Focal species (FS) are intended to be protective for other species based on their exposure (e.g. food intake rate, diet composition, etc.) and are therefore used for higher tier field studies to refine other factors of the risk assessment (e.g. PT and PD). In the updated draft Birds & Mammals Guidance Document from 2021, FS are defined as “a representative subset of real species that actually occur in the crop when the plant protection product is being used”. FS are usually selected based on their ecological relevance and their likely exposure (and thus risk) to the potential stressor under field conditions. Focal species for higher tier risk assessment are usually determined in field studies in which bird surveys are conducted in the relevant crop and at the relevant BBCH stage for the risk assessment. According to the currently valid EFSA Guidance Document for Birds and Mammals Risk Assessment (2009), species observed with a frequency of occurrence (FO, ie. the percentage of study fields in which the species was observed) of more than 20% would be of high priority especially if they also have high dominance (ie. the species makes up a relatively large proportion of the overall avian population present in the crop). Other factors to be considered in the focal species selection are feeding strata, food intake rate, body weight and diet composition. The draft guidance mentions that for FS selection the frequency of occurrence is not as important as feeding guild, body weight and diet composition and species with a low frequency of occurrence (< 20%) should not be automatically eliminated from focal species selection. Instead, a species with high potential dietary exposure which less frequently observed, may be considered more appropriate for the risk assessment. It may also be more protective and better cover similar species, than other species with higher occurrence. Clear guidance on how this process should look like is missing. Consequently, there is a high risk that future FS selection will lack a harmonized approach and moreover might result in selection of less exposed species for the higher tier risk assessment. We propose a harmonized method for focal species selection. For this purpose, some existing industry owned focal species studies will be selected and the FS will be re-assessed following the recommendations given in the draft guidance. Based on these examples a harmonized approach will be proposed for future FS selection.

6.02.P-We249 Improved ECx/BMDx Estimation for Avian Studies

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To inform regulatory authorities, to better understand the capabilities and limitations of benchmark dose (BMD) methodology, and to make a recommendation on improved experimental design for the BMD approach, a computer simulation study was conducted to explore the BMD methodology using model averaging applied to generalized non-linear mixed models (GNLMMs) for conditionally binomial and Poisson endpoints that are required in avian reproduction studies. Among the advantages of GNLMM over traditional normality-based approximate methods is the elimination of negative lower confidence bounds. More importantly, these models more accurately reflect the nature of the observations. The work explored alternative experimental designs with different spacing (e.g., common ratio of test concentrations 4 or 6 as well as concentrations equally spaced on an arithmetic scale (rather than geometrically spaced) and different replication with the goal of finding practical experimental design better suited for regression/BMD methodology without unduly affected NOAEL calculation when needed. Power simulations were also done to determine the impact of the alternative experimental designs on NOEC determination. The work also addresses the different models and model selection criteria used by existing software packages to implement BMD methodology and their impact on BMDx and BMDxLB estimation. These packages include both R and Excel-based software and both Bayesian and non-Bayesian methodology. A summary of the benefits of the work follows. • Fully addresses the statistical protocol proposed in Green *et al* (2021) • Eliminate the potential for mis-applying normality-based methods on non-normal data • Inform the most useful experimental design for BMD estimation • Compare NOAEL to EC10 & EC20 to help risk assessment when no BMDx can be estimated

6.02.P-We250 Development of a Bioenergetic Model for Improved Analysis of Avian Reproduction Studies

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Current environmental risk assessments addressing chronic exposure of chemicals to birds are based on complex and controlled laboratory reproduction experiments. These experiments produce many response variables that are usually separately analysed which can hamper understanding of the physiological mechanisms underlying the effects of the tested chemical as well as extrapolation to field conditions. In addition, behavioural responses are rarely considered. Here we posit that a better way to evaluate avian reproduction study data is to use mechanistic modelling, such as Toxicokinetic – Toxicodynamic (TKTD) modelling. Specifically, Dynamic Energy Budget (DEB) models coupled with TKTD modules (DEB-TKTD) are the leading approach to assess sublethal effects of chemicals on individuals by more comprehensively using data available through integration in a unique framework. Existing DEB models do not include the necessary flexibility to accurately capture the variety and specificity of data from avian reproduction studies. Here we present a tailored DEB-TKTD model able to overcome this challenge. We tested our model on 10 avian reproduction studies conducted on 5 different pesticides. The new implementation accurately captured the growth and reproduction observed in these studies. In addition, with this implementation, it is possible to disentangle and to quantify possible behavioural effects from direct toxic action of the pesticides. This approach has the potential to make better use of existing and future data sets and provides means for a more accurate extrapolation of effects to birds from pesticide exposures in the field.

6.02.P-We251 How to Derive Realistic Foraging and Food Consumption Pattern for Use in TKTD Models for Wildlife Risk Assessments?

Dirk Nickisch, Imme Katzschner, Felix von Blanckenhagen, Jens Schabacker and Ludwigs Ludwigs, Rifcon GmbH, Germany

Following the EFSA Wildlife draft guidance published end of 2021, toxicodynamic-toxicokinetic (TKTD) models have been suggested as Tier 2 refinement for bird and mammal risk assessments of pesticides. In recent years, TKTD models supported the aquatic risk assessment, and first experience were made in soil risk assessments. An important TKTD application in the aquatic risk assessment is the prediction of toxic effects under realistic, time-varying exposure. So far, in Wildlife risk assessments the TK related and species-specific body burden modelling was applied for higher tier weight of evidence assessments particularly for certain mammalian scenarios and based on previous negotiations with member state authorities. Realistic exposure profiles using version-controlled EU modelling tools for aquatic or soil exposure profiles are established in these areas in ecotoxicology. However, the application of TKTD models in Wildlife risk assessments seems practically limited due to the lack of realistic food intake patterns. Wild animals need to feed during large parts of the day (and/or night) to cover energetic requirements within 24h hours. Therefore, toxins are not ingested in a single dose but in multiple small doses in complex feeding patterns. No accepted tools are available, and more realistic foraging pattern of individuals in farmland like the PT (portion of diet from treated area) refinement is part of step 3 of the tiered effect assessment proposed by EFSA in 2021. Here, we present and compare different approaches to estimate foraging and food intake patterns, using the example of the rabbit. We derived patterns from a) literature data, b) GPS satellite tracking data and c) VHF-technology related radio-tracking fieldwork. We tested the different patterns in TKTD models with substances, which are metabolized at different rates in the animal's body. Furthermore we discuss what is needed to improve the possibility to apply TKTD models also in Wildlife risk assessments, and ask to establish a joint way forward implementing this approach as part of the higher tier risk assessment for terrestrial vertebrates.

6.02.P-We252 How to Use Mechanistic Effect Models in Bird and Mammal Risk Assessment Without Additional Vertebrate Testing

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Recently the draft of the EFSA bird and mammals guidance document was published for public commenting. The criteria of the EFSA scientific opinion (SO) on TKTD models for model calibration and validation are cited. According to this EFSA SO (EFSA PPR Panel, 2018) TKTD models must be calibrated based upon experimental data for the specific species-pesticide combination, and subsequently validated on an independent dataset with a different exposure situation to prove that the model is able to extrapolate across different exposure conditions. Unfortunately, this calibration and validation exercise would increase the number of animals tests. One of the aims of the Regulation (EC) No 7441107/2009 is to reduce vertebrate testing. Therefore other ways for model calibration and validation should be used. An option would be to use a combination of *in vitro* assays and toxicokinetic modeling to predict the responses from the standard tests. In that case the species-pesticide specific calibration is conducted with *in vitro* assays for the toxicodynamics and ADME studies for the toxicokinetics and the standard animal studies are used for model validation. Examples of such an approach for lethal and sublethal effects are available in literature. By use of physiological based toxicokinetic models, with the addition of *in vitro* studies it might also be possible in future to conduct the calibration on one species (e.g. rat) and the validation on an independent one (e.g. mouse or quail). This is currently not established and needs further research. We will present an overview of this alternative approach and examples for the different steps.

6.02.P-We253 Comparison of Four Methods of Sampling Nocturnal Flying Insects and Their RESIDUE Results

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The official update process of the Guidance Document (GD) on the risk assessment (RA) for birds and mammals provides a great opportunity to present developments regarding the collection of data linked to the exposure components of the risk assessment. Crucial for the exposure estimation are the residue decline field studies. The exact methods used to sample the test material, whether arthropods or plants, and their practical application, can differ due to various reasons. Therefore, a systematic comparison among methods should improve our understanding of how these can impact the outcome of such studies. The arthropod group of flying insects represents the link to the potential oral exposure for insectivorous birds and mammals (for the later, especially those with nocturnal behaviour). So far there is no consensus about the best sampling methodology for obtaining data to perform residue analyses on that group. Additionally, information on the comparison of methodologies for trapping flying insects is scarce with respect to the measured residues after pesticide application in agricultural areas. The study aimed to determine a reliable and representative sampling method for performing analyses of residue values of food items (nocturnal flying insects) in common agricultural habitats. Four different methods (light trap, Malaise trap, aspirator and quad-netting) to capture nocturnal aerial insects were tested in two different crops (vineyards and orchards), following the application of two fungicides with Tetraconazole as active ingredient, in the southwest of France during summer 2020. The samples were analysed for residue values RUD (Residue unit per dose). The results allowed the comparison, for each method and habitat, of the sample composition and of the observed residue values and provide the basis for the discussion about the influence of insect community composition in the residue results. This study provides a good example of method comparison, which could be usefully extended to other cases of arthropod sampling for residue studies (pitfall sampling, beating, inventory spraying, sweep netting, etc.).

6.02.P-We254 Industry Data Contribution to EFSA for the Update of the Guidance on Risk Assessment for Birds and Mammals

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We present examples of Industry data contributions for the update of the EFSA Guidance on Risk Assessment for Birds & Mammals, and how they were evaluated by the EFSA Working Group: Residue levels of pesticides on fruits for use in wildlife risk assessment. The fruit RUD values in the 2009 EFSA GD were based on a published compilation of various trials of unclear regulatory relevance. Therefore, Industry offered EFSA a dataset of field study data on fruit residue levels from applications of pesticides in fruiting crops. The revised RUD values adopted by EFSA in the 2021 draft GD were derived by merging this Industry dataset with data from the 2018 Lahr et al report. Dissipation of plant protection products from foliage. The 10d default foliar dissipation DT₅₀ value in the 2009 EFSA GD was based on a compilation of residue trials no longer relevant for current pesticide use in the EU. Therefore, Industry proposed to consider the Ebeling & Wang evaluation on regulatory residue trials, from which DT₅₀ values of 3.2d (geometric mean) and 7.9d (90th percentile) were derived. Based on this and other available publications, the 2021 draft GD acknowledged the need to evaluate the adequacy of the 10d value, but this was not conducted due to time limitations and the conservative 10d default DT₅₀ value was maintained. Extrapolation factors for LD₅₀ values from acute mammal studies conducted at the limit dose. The 2009 EFSA GD provided guidance on how to extrapolate a surrogate LD₅₀ for birds, but not for mammals. Industry provided EFSA with an evaluation and a dataset of mammalian acute toxicity studies to derive suitable extrapolation factors. From a broader dataset, EFSA conducted a parallel evaluation which resulted in extrapolation factors for mammals being established and included in the 2021 draft GD. Time-Weighted Average (TWA) In mammalian toxicological studies used for wildlife ecological risk assessment, the duration of exposure is fixed and not adjusted to the expected exposure duration in the field. However, time dependency is a typical feature of toxicity: the longer the exposure, the greater are the effects. In the 2009 EFSA GD a default 21d TWA window to calculate exposure is used. Industry provided an analysis demonstrating this is an overly conservative approach when bodyweight effects after much longer exposures are used for wildlife risk assessment. In the 2021 draft GD, EFSA acknowledged uncertainties that must be considered for those cases

6.02.P-We255 Reducing Exposure: Comparison of Two Application Techniques of a Granular Pesticide in Potato Fields

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Granular plant protection products (PPP) are widely used and have potential to cause adverse effects to birds and mammals if ingested in sufficient quantities by individual animals. By minimising the availability of granules on the soil surface, the risk of exposure can be reduced to levels that pose a low risk to birds and mammals foraging within treated fields. This study was performed to compare two common application methods - in-furrow application and broadcast - for a granular PPP used in potato cultivation, to assess the number of granules remaining on the soil surface following application. Exposure assessments were carried out on potato fields in four European countries by counting the number of granules on the soil surface for a defined number of plots on each of the study fields directly after application. The number of exposed granules was generally lower on fields with in-furrow applications than on fields where broadcast application was used. Thus, in-furrow application of granular PPP can mitigate the risk of exposure to wildlife potentially ingesting granules left on the soil surface.

6.02.P-We256 EMBARC: To Elucidate the Risks to Bats From Exposure to Agrochemicals

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Intensification of agriculture has been identified as a key driver for bat population declines, with the associated exposure to legacy agrochemicals being directly linked to mass mortality events in bat populations (e.g. organochlorines). This has led to a general assumption in the scientific community that bats are exposed to agrochemicals and that these could lead to adverse effects on individuals and populations. This is reflected in the recent European Food Safety Authority (EFSA) Scientific Opinion that concluded that in some instances, the risks to bats were not adequately covered by current risk assessment requirements in the authorisation of Plant Protection Products under EC 1107/2009. However, significant data gaps have been identified when trying to quantify routes of exposure, sensitivity to agrochemicals and relevance of effects on bat populations (e.g. Brooks *et al.* 2021). A newly funded project entitled “*Exploring Mayflies and Bats: Assessing Risks from Chemicals (EMBARC)*” will fill some of these data-gaps incorporating fieldwork and effects modelling approaches. Three seasons of bat fieldwork will be undertaken at two Newcastle University Farms monitoring species richness and activity to identify suitable focal species for a range of crops across different growth stages that may be used as a basis for a quantitative risk assessment methodology similar to that currently used for birds and mammals (EFSA 2009). Further field studies will be performed to identify whether different types of disturbance associated with agrochemical application (e.g. noise, artificial light, spray) affect bat activity and ultimately exposure. In conjunction with fieldwork, population models will be developed to explore whether sub-lethal effects of agrochemicals may have more subtle effects on long-term population stability. This project will provide a greater level of understanding of the routes of exposure to bats and how to incorporate this information in a regulatory risk assessment. Furthermore, we will have a better understanding of population responses to agrochemical exposure and how different strategies or agronomic practices may be implemented to mitigate any adverse effects.

6.02.P-We257 Possibilities and Limitations for the Bridging of Focal Species Between Different Countries

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Plant protection products (PPPs) are applied to specific crops at defined growth stages and during predetermined periods of the year. In order to assess the risk of exposure of birds and mammals to these PPPs, so called ‘focal species’ can be identified. It is the aim of the ‘focal species’ concept, that one (or a few) species cover all other species of the same feeding guild, in the risk assessment that forage on PPP treated fields. A common approach to identify focal species is the implementation of bird and mammal surveys on several fields of the respective crop during the period of potential exposure to the PPP. These surveys are commonly conducted in one region in a single country only, while the PPPs can be applied to the same crop in many different countries. In order to assess the risk to birds and mammals by the PPP in these other countries, a common approach is the so called ‘bridging’. That means that the focal species identified for one country are used to refine the risk for birds and mammals in other countries, too. However, there might be factors that restrict the applicability of this approach. For example, the conditions where and when crops are cultivated or the habitat preferences of the selected focal species might differ between countries. In addition, the geographical distribution of birds and mammals may limit the occurrence of species to certain regions. Hence, the bird and mammal community present on fields of the same crop and at the same growth stage might differ between countries, even within the same regulatory zone. In order to illuminate the possibilities and limits of the ‘bridging’-approach, data on the bird and mammal community in a certain cultivation stage of a field (i.e. ‘bare soil’) obtained in four different countries (two countries in two regulatory zones, respectively) were examined for similarities and differences. This poster will present the results and recommendations of this analysis.

6.02.P-We258 The 'Portion of Diet From Treated Area' As Robust Refinement in Pesticide Risk Assessments for Birds and Mammals in Europe - History and Update

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The risk quotient based long-term exposure assessment to pesticides for birds and mammals in Europe can be refined by including more realistic exposure field data on the relevant focal species in the relevant crop. One of the most frequently used refinement

factors in the long-term risk assessment is the PT factor, intending to reflect the proportion of the diet taken from the pesticide treated area. PT field data are obtained by tracking individual animals for a single or few daily activity periods. An overall PT factor to be used in the risk assessment is then determined based on a number of individual PT values. In 2017 Ludwigs et al. proposed determining overall PT factors by including inter- and intra-individual variability in PT within the given long-term period in order to obtain a more realistic long-term PT value. The basic principle of this approach is to calculate 21-day mean PT values of individuals followed by a 90th %ile PT factor for a virtual population based on solid PT field data sets using Monte Carlo simulations. The approach by Ludwigs et al. was critically reviewed by Crocker & Langton (2019). Concerns have been raised that the method proposed is unsuited to most radio-tracking datasets, statistically not valid and could lead to misleading and possibly not protective risk assessments. As a follow-up on this discussion, datasets of yellow wagtails (*Motacilla flava*) in strawberries and skylarks (*Alauda arvensis*) in spring cereals were analysed to demonstrate applicability and limitations of the proposed method. With this exercise, we aim to further contribute to the discussion on how to derive protective PT factors for higher tier and long-term risk assessments.

6.02.P-We259 Automated Radio-Tracking in the Risk Assessment for Birds and Mammals Under Pesticide Legislation

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The official update process of the Guidance Document (GD) on the risk assessment (RA) for birds and mammals provides a great opportunity to present developments aimed to improve the collection and interpretation of data related to the exposure components of the risk assessment. Radio-tracking is a well-known, highly accurate methodology for the monitoring of a number of individuals. In the context of risk assessment, data obtained with this method is primarily used to estimate the Proportion of an animal's daily diet obtained in a Treated habitat (PT) and therefore as a refinement option for higher tier assessments. However, manual radio-tracking as currently prevailing method implies a high manpower need, relatively high costs and a relatively reduced number of monitored individuals in the end. The alternative use of GPS technology is restricted to larger animals, and therefore not operative with, for instance, small songbirds. Within this scenario, the technology of automated radio-tracking as a new, automatic system for recording movement and position of small animals, could be used to generate extensive and valuable information for the higher tier assessment step. This open-source project has developed a system for the monitoring of movement profiles of small wild animals, with emphasis on the ease of system use. The system characteristics include, in theory, a significantly increased precision and the unique possibility to monitor several animals at the same time and during long periods (as long as the transmitters work and birds stay within the monitoring grid) with manageable effort. Originally, the method was developed for bat monitoring. We started trials to test the feasibility of this approach for avian species (and, in the future, for small mammal species). The aim was to learn more about the system itself, the quality of the data collected and the possible technical setbacks or modifications required for birds. Several individuals of three small songbird species were equipped with a radio transmitter, in an area where a number of automated stations were installed. The respective radio signals were recorded by the system, and in parallel the crop development in the study area was monitored. The joint analysis of these data shows the potential for future use of this novel approach in the context of higher tier assessment, and offers a first insight on its advantages and disadvantages with respect to the manual radio-tracking.

6.02.P-We260 Validation and Application of a Non-Invasive Method to Monitor Farmland Bird Exposure to Triazole Fungicides

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The treatment of seeds with pesticides is an extended practice in current agriculture. In particular, triazole fungicides are the most widely used products to treat rainfed cereal seeds, so there is a high risk of exposure of granivorous bird species, such as the red-legged partridge (*Alectoris rufa*), that can consume those seeds remaining on the field surface during sowing. Triazole fungicides can cause endocrine disruption and reduce the reproductive capacity of partridges, which in the long run could result in population declines. This work aims to (i) develop and validate a non-invasive method to detect the presence of triazole fungicide residues in farmland birds by analyzing fecal samples of experimentally exposed red-legged partridges, and (ii) use the method in a field scenario to assess exposure of wild farmland birds. To this end, we exposed adult partridges to seeds treated with two formulations containing three triazoles as active ingredients (flutriafol, prothioconazole, tebuconazole). We determined the concentrations of the three triazole fungicides and their main metabolite (1,2,4-triazole) in two types of feces (cecum and rectum stool samples) at three different times: immediately after exposure, and on days 7 and 14 post-exposure. To validate the method in a real scenario, we collected a total of 118 fecal samples from wild farmland birds. The results show that the three active ingredients and 1,2,4-triazole were only detected in samples collected at the end of the exposure, not afterwards, indicating that fungicides were rapidly excreted in feces. The presence of triazole fungicides was detected in 43% of rectal stool samples and in 37% of cecum samples from exposed partridges. The metabolite 1,2,4-triazole was detected in 53% of the rectal samples of exposed partridges. The analysis of feces from wild farmland birds found detectable levels of triazole fungicides in 20% of samples. The results of the experimental exposure study indicate that this is a minimum, probably underestimated, exposure level for wild birds. Our results reinforce the need to develop effective and apply non-invasive methods to monitor the real exposure of farmland birds to pesticides in the field.

6.02.P-We261 Higher Tier Field Effect Studies in Practice and the Benefits and Drawbacks of MDDs to Evaluate Them

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The proposed new EFSA Guidance Document on risk assessment for birds and mammals strengthens the use of higher tier field effect studies. The draft document describes furthermore the use of Minimum Detectable Differences (MDD) as a viable option for a statistical assessment of a study. We provide MDDs from two different field effect study designs on mammals and discuss a way to evaluate them. Other than a statistical power analysis, MDDs apply only to the one study they are calculated for. Due to different focal species, seasons, years or locations the MDDs of different studies vary a great deal. We calculated the variation of untreated controls within each study to find a benchmark MDD, assuming that any detection level within the natural variation between untreated controls is sufficient to detect any adverse effect of biological importance. We are fully aware that the derivation of an evaluating benchmark from data within a study needs further consideration. Furthermore, the assumption that any effect smaller than the natural (from control sites) variation of a parameter is of no or little biological importance for the population as such will and should not be accepted without further discussion. However, a meta-analysis of control field data instead of the data from just one study may help to strengthen MDD benchmarks. The definition of a set of MDD categories instead of a single benchmark value will help to specify the protective level of the study results.

6.02.P-We262 How to Jump Pitfalls in the Statistical Analysis of Field Effect Studies

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Field effect study data holds certain pitfalls that have to be taken into account in the analysis as they affect the choice of the statistical method. We draw attention to some of these pitfalls and show possible approaches on how to jump them. Especially, as the draft guidance on birds and mammals risk assessment using higher tier studies does not include approved methods for the analysis of field effect studies. However, there are several appropriate ways to analyse this kind of data statistically. We use the concept of Generalized Linear Mixed Models (GLMMs) to give one option that is able to cope with many of the peculiarities of field effect study data. A first step is to know what kind of data one is dealing with. The numbers itself characterize the underlying data type and distribution. Most data in ecology are Poisson-distributed count data. However, there are other data types such as proportions or ratios. Most statistical methods make assumptions about the data type they are used on and therefore, method and data type should fit together. When applying GLMMs, the distribution can be specified according to the data type. In field effect studies, the data is usually replicated. If a replicate is observed or measured more than once, the data is dependent. Some statistical methods assume independence of the data and are thus not suitable in these cases. GLMMs are made to cope with independence using the concept of 'random effects'. Data exploration is a crucial step in analysing any kind of data. It intends to familiarize oneself with the data and get to know its limitations. It includes the investigation of outliers, zero observations, and correlation between covariates. GLMMs provide options to handle such cases as overdispersion and zero-inflation. They also provide the opportunity to include more than one explanatory variable. This can improve the fit of the model to the data, make other effects easier to detect, and allows to look at effects over time by including interactions between variables. However, explanatory variables should not be correlated and overfitting should be avoided. Validation of fitted models is important to verify that assumptions, such as independence and absence of residual patterns, are not violated, and should accompany the statistical results. If applied correctly, GLMMs are a powerful tool that provide a way to analyse field effect study data and that can handle many of the obstacles that come with ecological data.

6.02.P-We263 Methods for tier3 Bird Mammal Field Studies Beyond the Existing and Upcoming EFSA Guidance Document

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Within the existing and the proposed new EFSA Guidance Document on risk assessment for birds and mammals a variety of different methods for the conduct of field studies with birds and mammals are mentioned. In the first draft of the 'new' guidance document many aspects of field study methods are further specified and are summarised as tier 3 in the stepwise approach of the risk evaluation. The draft also gives applicants better guidance how methods should be used (and which limitations specific methods may have). Nevertheless, more methodological approaches for field studies were developed within recent years and can broaden and enrich the toolbox for tier 3 studies (field exposure studies and field effect studies). Examples will be given for a set of different methods like the 'giving-up-density' (GUD) approach, use of cameras in field studies and automated systems to verify the survival and activity of individually marked animals by 'passive-integrated-transponders' (PIT's) - without re-trapping the animals. Those approaches can be used as stand-alone methods in specific studies, as combined methods or as additional methods in studies as already defined in the guidance document.

6.02.P-We264 Taming the Wild - Reducing Uncertainties of Field Effect Studies in a Semi-Field Set-Up

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Under the current EFSA Guidance Document on Birds & Mammals Risk Assessment the long-term risk assessment of pesticides for small herbivorous mammals (voles) fails on many occasions. The commonly accepted higher tier focal species is the common vole (*Microtus arvalis*). Higher tier approaches refining ecological and species-specific data generally do not significantly improve the Tier I risk assessment due to the general ecology of this species. Therefore, a more common higher tier refinement for small herbivorous mammals are field effect studies, designed to monitor population effects for populations exposed to a certain pesticide and to compare the recorded data to populations not exposed. However, such guidance-recommended field effect studies

conducted to investigate assumed effects of pesticides on small mammal communities are regularly challenged by the evaluating authorities, because these studies are conducted in open systems (i.e. including immigration, emigration, predation etc.). These factors are believed to increase the uncertainty of the outcome of such a field effect study. Consequently, authorities partly question these field study designs and consider them inadequate, even if untreated control communities, investigated at the same time, do not show any statistical differences to the treated subpopulations. In order to address these concerns, Rifcon GmbH established an experimental set-up for semi-field studies in enclosures to address long-term effects on small herbivorous mammals after exposure to a pesticide. The general set-up of these enclosures was presented at the SETAC Europe conference 2017 in Brussels/Belgium. Here, we present individual-based data from recently run enclosure studies demonstrating the functionality of this system in relation to the risk assessment.

6.02.P-We265 Grassland Fields As Surrogate Crop for Field Effect Studies on the Common Vole

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The common vole occurs in all countries of the central regulatory zone (except Ireland) and in some countries of the northern and the southern zones too. The species can be found in many crops and during its population outbreaks it even becomes a pest. Yet, the common vole does usually have no permanent populations on arable fields due to farming practices like ploughing, harrowing or harvest. Permanent grassland fields used e.g. for hay production, provide a far more stable habitat that sustains vole populations for a long time. For long-term studies to investigate chronic risks from PPPs, it is essential to examine at least one entire reproductive season. Grassland fields provide another advantage, as they are not treated with PPPs. Consequently, a field effect study in grassland can be conducted with 'clean' control fields and with treatment fields that are only treated with the test item but no additional PPPs. Spray applications on short grass with the same equipment that is used on arable fields provide a realistic worst-case exposure. As common voles have quite small home ranges and feed almost exclusively on the grass- and broad-leaved vegetation, their exposure by food intake is close to 100%. Voles are easy to capture and frequently recaptured after marking and, thus, providing an ideal species for capture-mark-recapture (CMR) study designs. The design features stable small mammal populations that are restricted to a full sized field by habitat preferences and behaviour, and of sufficient population density to obtain statistically robust results. It allows the use of a single test item to be compared to a 'clean' control. The concept of surrogate crop as a valid study design might even be extended to other habitat-focal species combinations.

6.02.P-We266 Review of Approaches Taken for "Vole" Risk Assessment for Plant Protection Products Amongst European Regulatory Authorities

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There is a clear lack of harmonisation in the approaches to higher-tier bird and mammal risk assessments taken by European Regulatory Authorities for Plant Protection Products (PPP). Here we focus on the different approaches taken for the small herbivorous mammal (represented by the "vole"). We have reviewed national approaches, either from published national guidance or contacting the Regulatory Authorities directly. We present a summary of our findings, including the scale of the different approaches taken. The common vole (*Microtus arvalis*) is defined in EFSA's Bird and Mammal Guidance Document (2009), as the representative species for the small herbivorous mammal. The first-tier risk assessment for the small herbivorous mammal is highly conservative and often fails. EFSA's Guidance allows for a number of different refinement options for use in a higher-tier risk assessment. This includes defining the focal species, i.e. a real species that occurs in the crop when the pesticide is being used. Following an EU level assessment/review of a pesticide active substance (under Regulation (EC) No. 1107/2009), refinements including determination of a focal species are often left as an open point to be defined at Member State (MS) level during product authorisation. Some MSs (e.g. Greece and France) simply consider the small herbivorous mammal ("vole") irrelevant, due to its pest status and high fecundity. As such, the risk is covered by assessments for other mammalian generic focal species. Other MSs (e.g. Germany and Czech Republic) allow the reduction of the trigger value, where the toxicity endpoint used for the assessment has been determined for phylogenically related species. The field vole (*Microtus agrestis*) is considered more relevant than the common vole by some MSs (e.g. Northern Zone MSs and UK). Whereas some MSs (e.g. Belgium) require field studies in the crop of interest if the Applicant wishes to refine the focal species. Overall, there is a wide range of approaches taken by different MSs in assessing the risk to the small herbivorous mammal "vole". This ranges from perceiving the vole as a pest species, to considering the vole as a species to be protected, and the need for higher-tier data to support the risk assessment. One of the aims of the revision of EFSA's Bird and Mammal Guidance was to further harmonise higher-tier assessments with regulatory zones, however, the draft revision (2021) does not address these MS approaches.

6.02.P-We267 Secondary Poisoning of Birds and Mammals Via Benthic Invertebrates: Initial Evaluation of This Pesticide Risk Assessment As Found in the Draft EFSA Birds and Mammals Guidance (September 2021)

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The EFSA (European Food Safety Authority) guidance document for assessment of risk from pesticides to wild birds and mammals (2009) is currently undergoing revision. A draft revised document for public consultation was issued in September 2021. This draft includes revisions to existing risk assessments, as well as some new risk assessments. Risk of secondary poisoning of wild birds and mammals via consumption of earthworms and fish has been included since the original version of the EFSA guidance (2009); and was included in the pre-EFSA guidance too (SANCO/4145/2000, September 2002). In the 2021 draft, a new route of secondary poisoning has been proposed: via consumption of benthic sediment-dwelling invertebrates. We will evaluate this proposed new risk assessment, consider the types of compounds likely to trigger it, the calculations required, the results, and the consequences. We will illustrate our findings using example data sets.

6.03 Environmental Risk Assessment for nanomaterials – challenges and solutions for knowledge transfer and active dialogue between research and regulation

6.03.T-01 Updating the OECD 211 *Daphnia magna* Reproduction Test for Use With Engineered and Anthropogenic Particles

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The goal of standard hazard testing is to facilitate comparison of chemicals under identical conditions for ranking of toxicity to the test organisms, and as such environmental realism is not typically of concern. However, the highly reactive surfaces of nanoscale materials means that they acquire a corona of biomolecules from their surroundings which reduces their toxicity; in the absence of suitable biomolecules in the exposure medium the nanomaterials strip biomolecules from organismal membranes, leading to excessive damage and overestimation of toxicity. Exposure of the indicator species *Daphnia magna* to nanomaterials may lead to reduced growth, delayed maturation and delayed reproduction, induction of males and epigenetic changes passed from exposed mothers to their offspring. The current OECD test guideline looks at only a single generation, thus ignoring the possibility of impacts in subsequent generations, through adaption to continued exposure or sensitisation resulting from parental exposures with consequences for population dynamics. Recommendations for adaption of the OECD 211 for testing of nanoscale particles are presented, grouped into three broad categories: 1 Adjustments to exposure conditions and duration, to allow for delays in maturation and brood timings, use of daphnia-conditioned media to disperse the particles and passivate the particle surfaces prior to exposure to the organisms, monitoring of the induction of males and the presence of fertilised eggs, and testing both freshly dispersed and medium-aged particles. 2 Addition of particle-specific analyses to support determination of mode of action, including quantification of uptake dose before and after depuration, imaging of phenotypical changes and sub-cellular localisation of nanomaterials in the daphnia gut. 3. Extension to additional generations and inclusion of additional stressors (e.g., temperature) to explore epigenetic effects of environmental exposures, including the potential for adaption to nanomaterials exposure and/or increased sensitization to nanomaterials of organisms whose parent or grandparent generations had been exposed to nanomaterials. This would require multi-generational assessment, over at least 2-3 generations. As part of the EU Horizon 2020 project RiskGONE, these recommendations are being submitted to the Working Party on manufactured nanomaterials (WPMN) for consideration as a standard project proposal to update the OECD 211 Test Guideline.

6.03.T-02 Similarity Assessment of Metallic Nanoparticles

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Similarity assessment is one of the means of optimally using scarcely available experimental data on the fate and hazards of nanoforms (NFs) for regulatory purposes. For a group of NFs that are shown to be similar it is allowed in a regulatory context to apply the information available on any of the NFs within the group to the whole group of NFs. Obviously, a proper justification for such a similarity assessment is to be provided. Within the context of exemplifying such a justification, a case study was performed aimed at assessing the similarity of a group of spherical metallic NFs that differ with regard to chemical composition (three metals) and particle size (three different sizes). The endpoints of assessment were root elongation and biomass increase of lettuce (*Lactuca sativa* L.) seedlings and exposure assessment was performed in order to express the actual exposure concentration in terms of time-weighted average particle concentrations. The results of the study show that for the specific endpoints assessed, chemical composition is driving NF toxicity, and this is mostly due to impacts on the fate of the NFs. On the other hand, particle size of Cu NFs had a negligible impact on the dose-response relationships for any of the endpoints assessed. It is thus concluded that hazard data available on any spherical Cu NF can be used to inform on the hazards of any spherical Cu NF within the size range of 25 – 100 nm. Also, toxicity data for the Cu²⁺-ion are suited for such a similarity assessment.

6.03.T-03 Assessing the Similarity of Nanoforms Based on the Biodegradation of Organic Surface Treatments

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Understanding how modulation of key intrinsic properties of nanoforms, such as different surface treatments, affects exposure and hazard is a priority to address for developing grouping approaches. One hypothesis to group nanoforms with different organic surface modifications is to use parameters linked to the biodegradation of the surface treatment, where demonstrating loss of the organic surface treatment indicates it will no longer drive the fate and toxicity of the nanoform and so the nanoform may be grouped with other non-coated analogous forms. To this end, a tiered testing strategy consisting of a 48 hour colorimetric based screening assessment and a higher tier OECD ready biodegradability test (OECD TG 301F), is used to assess the similarity in biodegradation of 6 commonly used organic surface treatments for nanoforms. The pairwise similarity in biodegradation between substances using the data from the two tiers of testing is evaluated by Euclidean distance and maximal fold difference. The presentation will demonstrate a tiered testing strategy for biodegradability of organic surface treatments for nanoforms that may be employed within integrated approaches to testing and assessment (IATA), to group nanoforms according to their environmental fate and toxicity. Lessons learnt from pairwise similarity assessment inform thresholds for groups within each tier of testing, and

define the applicability range, wherein similarity assessment of biodegradability between nanoforms would be appropriate as part of a justification for grouping nanoforms with different surface treatments. Acceptable limits of similarity are yet to be determined, however approaches to validate limits of similarity will be discussed in the context of the six case study substances tested. This study has relevance for grouping and read-across approaches of nanoforms for both regulatory and safer by design purposes.

6.03.T-04 How Can We Justify Grouping of Nanoforms for Hazard Assessment? Concepts and Tools to Quantify Similarity at Relevant and Measurable Ranges

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To reduce the animal testing of *each* nanoform (NF), regulatory information requirements may be fulfilled by grouping approaches. The GRACIOUS Framework supports the user to identify a hypothesis, gather the data matrix by targeted testing, and to assess the similarity of the NFs within the proposed group. Algorithms quantify similarity, and thus compare the candidate group of NFs against each other and against representative test materials that represent the biologically relevant range of selected descriptors. Several approaches are accessible via a browser-based tool and are embedded in the blueprint of the e-tool implementation of the full framework. For the pairwise comparison of NFs we included: A Bayesian model assessment which compares two sets of values using nested sampling. A Arsinh-Ordered Weighted Average model (Arsinh-OWA) which applies the arsinh transformation to the distance between two NFs, and then rescales to biologically relevant threshold An x-fold comparison as used in the ECETOC NanoApp. Euclidean distance, which is a highly established distance metric. GRACIOUS Tier 1 methods typically are physical-chemical screenings or in-vitro assays, as suggested by ECHA guidance for a grouping justification. Similarity assessment is possible and required only within the overlap of measurable and biologically (environmentally) relevant ranges. The values of the data matrix can then be cropped to the relevant range before applying similarity algorithms. By a pilot interlaboratory comparison on all descriptors *defining* a nanoform (size, composition/impurities, shape, surface area, surface treatment), we found e.g. that differences in size percentile D10 up to 34% should not be interpreted; differences in the main constituent elements up to 8% should not be interpreted, etc. The robustness of methods lags behind for descriptors of *interaction* (such as in-vitro, dosimetry, dissolution), and depends on current and future OECD guideline projects.

6.03.T-05 Marine Risk Assessment of a Reference Anti-Corrosion Nanomaterial: Contributions From the NANOGREEN Project

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Metallic corrosion is an unsolved problem that represents 3.5% of the worldwide Gross Domestic Product. It has been minimized through toxic corrosion inhibitors, such as 2-mercaptobenzothiazole (MBT), that are added into maritime protective coatings. Some of those compounds have been successfully immobilized in stimuli-responsive engineered nanomaterials, namely Zn-Al layered double hydroxides (LDH) that can release the entrapped active ingredient under specific conditions coinciding with the degradation phenomena. In particular, the innovative nanomaterial LDH-MBT has been submitted to industrial validation by a Portuguese manufacturer of nano-based additives and thus, the soluble and nanostructured forms of the corrosion inhibitor can end up in the marine environment during the lifetime of the coating. However, their toxic effects on marine organisms remain scarcely studied. Thus, the present study comprehends a marine hazard and risk assessment of MBT in both soluble and nanostructured forms, which will be critical for benchmarking purposes with the upcoming nanoproducts developed under the NANOGREEN project that aims at developing a new generation of sustainable anti-corrosion nanoadditives. For that purpose, ecotoxicological short-term tests were carried out on 15 marine species for which NOEC and L/E/IC₅₀ values were calculated. Then, hazard endpoints (predicted no-effect concentration) were derived, and the risk quotient (RQ) was calculated between the maximum measured environmental concentration (MEC) of MBT in worldwide oceans (0.0017 mg/L) and the estimated marine PNEC (based on the NOEC values) for both forms of MBT. In terms of results, MBT was toxic, very toxic or extremely toxic for 66% of the tested species (lowest NOEC_{MBT}=0.001 mg MBT/L). LDH-MBT was at least 2-fold less toxic than the soluble form for all tested species (lowest NOEC_{LDH-MBT}=0.02 mg MBT/L), being not toxic for 40% of the tested species. The immobilization of MBT into the engineered nanoclay can protect the marine ecosystem by decreasing 15-fold the MBT hazard. It was also demonstrated that MBT poses environmental risks for the marine compartment (RQ=15.5), while its nanostructured form seems to be a promising nanoadditive featuring low environmental risk (RQ=1.0).

6.03 Environmental Risk Assessment for nanomaterials - challenges and solutions for knowledge transfer and active dialogue between research and regulation (Virtual Only)

6.03.V-01 Effects of Exposure Route and Surface Coating on the Bioaccumulation of CdTe Quantum Dots in Fish

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Fish can be exposed to nanomaterials (NMs) in several ways, however, little is known about their bioaccumulation potential. Although OECD Test Guidance 305 describes the methods for bioaccumulation testing in fish via water and diet, testing using an aqueous exposure of NMs may not be feasible. Dietary route testing is especially useful to overcome stability issues. Moreover, dietary ingestion of NMs has been shown to be an important route for aquatic organisms, yet knowledge is limited. Bioaccumulation potential of NMs is likely influenced by several physico-chemical properties and the distinct exposure pathway, and thus it is important to take into account all these factors when evaluating the bioaccumulation of these materials. CdTe quantum dots (QDs) were selected for our studies, because limited information exists regarding their bioaccumulation potential in fish. To address specifically the influences of exposure route and surface coating on bioaccumulation, the uptake and depuration kinetics of two differently coated QDs in rainbow trout (*Oncorhynchus mykiss*) were assessed following exposure via water and diet. In the minimised aqueous bioaccumulation tests, fish were exposed for 28 days to 4 or 40 µg/L QDs followed by 14 days of depuration. Fish were sampled at days 0, 14, 28, 35 and 42 for Cd and Te accumulation in the whole body to derive bioconcentration factors (BCFs). In the complete dietary bioaccumulation assays, fish were exposed for 28 days to 10 or 100 mg/kg QDs followed by 28 days of depuration. Fish were sampled at days 0, 7, 15, 21, 28, 29, 31, 35, 42 and 56 for analysis of concentrations of both Cd and Te in the whole body to derive biomagnification factors (BMFs). Additionally, fish were sampled for tissue analysis at days 28, 29 and 56. Cd and Te concentrations in whole fish and tissues following exposure to CdTe QDs showed uptake, distribution to tissues as well as a slow and incomplete elimination following a 14 day (aqueous study) or 28 day (dietary study) depuration period. Higher levels of Te were measured in fish exposed using the dietary route, however it is eliminated from fish bodies to the same extent as Cd. Dietary Cd appears to have a slower elimination from the liver, gill and intestine compared to Te. The PEG and COOH coatings did not appear to have an influence on bioaccumulation potential, however a higher bioavailability of Cd was observed after the water exposure. BCF/BMF values indicate a low bioaccumulation potential of Cd and Te following CdTe QD exposure in fish. *Acknowledgement* - The authors thank H2020 project GOV4NANO 814401 for its funding.

6.03.V-02 James Bond and the DaNa4 Knowledge Base: Why Reliable Risk Communication of Nanomaterials and Advanced Materials Are Crucial

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In the recent Bond movie “No Time to Die” James Bond is fighting a supervillain who wants to kill millions of people with nanobots. To answer the questions whether or not this scenario is a threat for humans or the environment, the DaNa project is providing information about the safety of advanced materials including nanomaterials and related technological developments. For more than ten years, the DaNa webpage (www.nanoobjects.info) provides safety-relevant information on 28 advanced materials including nanomaterials with respect to human and the environmental health. Even though many publications of the toxicology of nanomaterial or advanced material have been published, misinterpretations and fake news are a constant challenge. Such misrepresentations have multiple causes, both on the scientist’s side as well as on the side of multipliers. Therefore, the DaNa knowledge base offers a library of novel materials which are carefully and critically examined. A team of ecotoxicologists, human toxicologists and materials scientist is selecting proper publications by using a literature criteria checklist. The scientific knowledge of the quality assured studies is then transformed into an easy-to-understand material text. Furthermore, the DaNa knowledge base providing FAQ to socially relevant topics like nanomaterials in corona vaccines. With respect to the proper risk communication the project is transparent in how information and methodology are selected. Last but not least nanomaterial news are shared on social media and request from citizens as well as journalists are answered to fight fake news. This poster gives an overview on our activities of science communication in the field of materials safety. We describe our strategy and methodology to deal with risk communication issues and highlight the lessons learned over ten years of science communication on nanomaterials by outlining key principles that can be applied to communication on advanced materials. And if you are now wondering about nanobots, just check out our recent article “Nanobots – Science or fiction?” *Acknowledgement: The funding for the DaNa4.0 project is provided by the German Federal Ministry for Education and Research (BMBF) under grant no. 03XP0282.*

6.03.V-03 Static and Dynamic Testing for Determining Dissolution Rates of Metal Nanoparticles

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Potential adverse effects of metal nanomaterial towards the environment are attributed to their release of dissolved ions. The dissolution rate is therefore important regarding the assessment of potential ecotoxicological effects of metal nanoparticles and often requested by the European Chemicals Agency (ECHA). OECD Guidance Document (GD) 318 was issued in 2020 and describes the static and dynamic testing of metal nanomaterials to assess their dissolution rates. The OECD GD 318 offers diverse possibilities for testing concerning, e.g., test item loading, sampling times and testing media composition. First, the OECD GD 318 static batch test was realized on basis of OECD GD 29 (Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media) and nano-forms of Zn, ZnO and TiO₂ were investigated. For metal analyses of samples taken at different times, separation of solid or particulate material from dissolved species was conducted by centrifugal ultra-filtration. While static batch

testing of nano-Zn and nano-ZnO yielded sound results, tests with nano-Ag exhibited a fast formation of dissolved species as well as a reaction with constituents of the OECD 29 test medium. Therefore, the dissolution rate could not be determined using the static batch test. According to OECD GD 318, a dynamic test should be performed as an alternative. Thus, an appropriate dynamic test setup was developed and successfully applied to determine the dissolution rate for nano-Ag.

6.03 Environmental Risk Assessment for nanomaterials – challenges and solutions for knowledge transfer and active dialogue between research and regulation (Poster)

6.03.P-Th195 Comparison of Dispersion Stability of Organic Pigments: In Environmental Media of TG-318 and in Media for Guidelines TG-210 (Fish), TG-211 (Daphnia) and TG-221 (Lemna)

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The TG 318 on “Dispersion stability of nanomaterials in simulated environmental media” was the first nano-specific test guideline, published 2017 [1] and it has been previously applied to test the dispersion stability of organic pigments. Here we explored a suggestion by GD-318 [2] and tested the dispersion stability of 10 organic pigments in dispersion media that are used for eco-toxicological tests described in the OECD 210, 211 and 221 guidelines. The observed dispersion stability was compared to the prediction from the TG-318 matrix of media. In the present study was shown, that: · Testing the dispersion stability in the actual ecotoxicity medium could be an option. The main factor influencing the stability seems to be the ionic strength of the media. All three media have pH = 7 to 8, but differ in ionic strengths ($I_{210} < I_{211} < I_{221}$). Almost all tested pigments had low stability in OECD 211 and 221 media, while the stability in OECD 210 medium varied between pigments. · The stability results in the eco-toxicological testing media correlate to the ones obtained with the media in TG-318 at similar pH and I , with NOM. However, testing the original 3x3 media matrix (0, 1, and 10 mM Ca, pH = 4, 7 and 9) could be unnecessary. When direct testing in the ecotoxicity medium is not preferable, one can consider a testing in the media from TG-318 at the relevant for the ecotoxicological test pH value, but with more discrete levels of calcium. · In addition to the UV/Vis spectroscopy, recommended by TG-318 for organic materials, the particle size distribution directly in the environmental medium was checked with the analytical ultracentrifugation (AUC) [3]. The observed size distributions allow to exclude the pigment dissolution in the tested media. Moreover, the distributions of all 10 pigments confirm the presence of agglomeration, especially in the OECD 211 and OECD 221 media. Thus, the analytical alternatives to the TG-318 method of settling at 1 g can be considered: the measurement of time-resolved mass settling at 10 g to 10,000 g by the AUC method provided consistent results.

6.03.P-Th196 Reproducibility of Methods Required to Identify and Characterize Nanoforms of Substances - What Is the Achievable Accuracy for Similarity Assessments?

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Nanoforms of a substance are distinguished from one another through differences in their physicochemical properties. When registering nanoforms of a substance under the EU REACH framework, five basic descriptors are required for their identification: composition, surface chemistry, size, specific surface area and shape. To reduce the burden of testing of multitudes of nanoforms with only slight differences in these basic properties, similarity assessment can be employed to justify structural similarity of nanoforms on individual physicochemical properties. However, similarity assessment requires an understanding of the achievable accuracy of available methods, to have confidence in whether measured or reported differences are indeed, real. To estimate the achievable accuracy of common methods to characterise nanoforms, we assess the reproducibility of six analytical techniques routinely used to measure these five basic descriptors of nanoforms. Assessment was performed on representative test materials to evaluate the reproducibility of methods on single nanoforms of substances. The achievable accuracy is defined as the relative standard deviation of reproducibility (RSD_R) for each method. Well established, routine methods such as inductively coupled plasma mass spectrometry for main constituent analysis (ICP-MS), BET (Brunauer–Emmett–Teller) measurements of specific surface area, transmission and scanning electron microscopy for size and shape and Electrophoretic Light Scattering for surface potential and isoelectric point, all performed well with low RSD_R , generally between 5 and 20%. Maximal fold differences were usually < 1.5 fold between laboratories. Applications of technologies such as thermo-gravimetric analysis (TGA) for measuring water content and putative organic impurities, additives or surface treatments (through loss on ignition), demonstrated poorer reproducibility, but still within 5 fold differences. This poster will outline the research need for estimations of achievable accuracy under realistic conditions, present the results from this study for each of the techniques assessed and provide a discussion on the implications of this research for current approaches to similarity assessment and grouping of nanoforms based on intrinsic physicochemical properties.

6.03.P-Th197 Standardizing Methodologies in Ecotoxicology: Adapting Technical Guidelines to Assess Engineered Nanoclays (LDHs) Toxicity to Daphnia magna

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The expansion of the nanotechnology market may lead to the release of nanomaterials (NMs) into the environment. Consequently, there is an increasing need to evaluate their potential risk to the environment and humans. However, the wide variety and distinct

physico-chemical properties of NMs make their environmental risk assessment very challenging. The standard guidelines currently available do not account for all the nano-specificities, leading to uncertainties regarding NM's hazard assessment. Zn-Al layered double hydroxides (LDHs) are anionic nanoclays with 20 to 40 nm in height, standing as an innovative NM-based solution for a wide range of medical, environmental, or industrial applications, such as corrosion and/or biofouling prevention, or eutrophication mitigation. Such NM properties lead to several challenges when developing strategies to assess their hazard. Considering this, the present study aimed to i) assess the influence of two different OECD-recommended exposure methodologies (serial dilutions of the stock dispersion vs. direct addition of NM powder to each concentration) in the ecotoxicological profile of Zn-Al LDH (bulk powder) to the freshwater species *Daphnia magna*; ii) use the preferred methodology to evaluate the toxicity of different Zn-Al LDH grain size powders (bulk powder, < 25, 25-63, 63-125, 125-250 and >250 µm) to *D. magna*. In the direct addition of NM powder methodology, Zn-Al LDH was weighted individually for all tested concentrations. For the serial dilutions methodology, a stock dispersion was made and used for the highest tested concentration, with the remaining concentrations being achieved by a serial dilutions method. The direct addition of NM to each concentration was deemed as the preferred exposure methodology as the results revealed less variability. For the second aim, the ecotoxicological tests involving the different Zn-Al LDH grain size powders were performed using this methodology. The results showed that different grain sizes caused different toxic effects in *D. magna*. Some of the used Zn-Al LDH grain size powders were less toxic compared to the bulk Zn-Al LDH (with high variability in grain size distribution), helping to advise the producer company on the more eco-friendlier granulometry for several applications. Moreover, our findings support Zn-Al LDHs as a less toxic option compared to other Zn nanoforms (e.g. ZnO). To conclude, this study provides important data to help harmonizing methodologies for standardized protocols for testing NMs hazard. In the future, interlaboratory testing should be performed to establish standardized guidelines for ecotoxicological testing with Zn-Al LDHs.

6.03.P-Th198 Reliable Algal Testing of Nanomaterials - Results From an Inter-Laboratory Study

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The algal toxicity test is one of three mandatory tests required for classification, labelling, and safety assessment purposes in European and international chemical and nanomaterial regulations. Algal testing of nanomaterials has traditionally faced a number of challenges affecting the reproducibility of test results and hence their regulatory adequacy. To address these challenges, we have previously developed a testing platform, LEVITATT (LED Vertical Illumination Table for Algal Toxicity Tests) within the EU project PATROLS. In the current study, the LEVITATT and a standard operating procedure (PATROLS SOP) compliant with OECD TG201 was tested for repeatability (intra-laboratory variability) and reproducibility (inter-laboratory variability). The latter was done in a round-robin test with five European laboratories as participants. The validity of results obtained in the round-robin testing was addressed by the inclusion of a reference toxicant (3,5 dichlorophenol) as one of the two test compounds. In terms of variability, the round-robin testing of 3,5 dichlorophenol was comparable to the ISO 8692 round-robin test (coefficients of variation 0.41 and 0.38 for the PATROLS and ISO testing, respectively). This, as well as comparisons of EC_{50,72h,-} values confirmed the validity of the PATROLS round-robin test. For the tested nanomaterial, CeO₂ (NM-212), a somewhat larger variability was found. While this was to be expected when nanomaterials are tested, the variability could largely be traced back to the method for biomass determination. When comparing test results using the method recommended in the PATROLS SOP, the variability of test results in the round-robin test obtained with NM-212 was in the same range as seen for the reference toxicant. Based on the above, it is concluded that the PATROLS SOP and the LEVITATT testing setup increases the reliability of algal toxicity testing of nanomaterials by introducing practically feasible methods and testing principles that have been documented to be in compliance with regulatory requirements.

6.03.P-Th199 Nanoclays (LDHs) As Eco-Friendly Nanomaterials: Testing Their Safety Towards Freshwater Species

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Layered double hydroxides (LDHs) are emerging engineered nanomaterials, having recently received a lot of attention from both industry and academia. LDHs are anionic nanoclays, composed of positively-charged layers with di- and trivalent metal cations, and stabilized by anions and water molecules in the interlayers. Their excellent anion exchange ability and memory effect are some of the outstanding properties that make these nanomaterials attractive for a vast field of applications, such as medicine, pharmaceutical, biotechnology, among others. Even though LDHs have been regarded as promising eco-friendly nanomaterials, their ecotoxicological effects towards organisms need to be tested to ensure their safety and regulation. In particular, there is a lack of studies on the ecotoxicity of LDHs to freshwater species, which is critical for regulatory purposes due to their applications for remediation of water bodies and antifouling paints. Considering this, the aim of this study was to assess the ecotoxicity of Zn-Al LDHs and Mg-Al LDHs (calcined and non-calcined), in several test model freshwater species: the microalgae *Raphidocelis subcapitata* (algal growth inhibition test), the zebrafish *Danio rerio* (fish embryo toxicity test), the water flea *Daphnia magna* (acute immobilization test) and the macrophyte *Lemna minor* (growth inhibition test). Overall, results showed that LDHs have very low toxicity to the species tested, with EC₅₀ > 100 mg/L to *R. subcapitata*, *D. magna*, *D. rerio* and *L. minor*. This study provides data that supports the environmentally safe application of LDHs and can be used for nanoregulations, contributing for their environmental risk assessment.

6.03.P-Th200 Evaluation of the Toxicity of Corrosion Sensing Coatings Lixiviates to Marine Microalgae and Crustaceans

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The project "SMARTAQUA" (MarTera ERA-NET cofund scheme of H2020) is developing an eco-friendly low-cost early corrosion detection tool which can be applied directly on maritime steel structures. Hexacyanoferrate ions $[\text{Fe}(\text{CN})_6]^{3-}$, which can coordinate with Fe^{2+} ions resulting from the corrosion of steel, giving a blue colour associated with the formation of Prussian Blue, were incorporated in layered double hydroxides (Mg-Al LDH). Entrapment of active compounds in LDH nanocarriers were found to render controlled release of $[\text{Fe}(\text{CN})_6]^{3-}$ ions under conditions relevant for corrosion processes. Fe_3O_4 magnetic nanoparticles were also developed for magnetic detection of corrosion. Then, materials were incorporated to two types of polyhedral oligomeric silsesquioxanes based formulations: LAPOSS 1 and LAPOSS 3. A total of 3 coatings per formulation were produced: a blank coating, a coating with 5wt% Fe_3O_4 , and a coating with 5 wt% Mg-Al/ $[\text{Fe}(\text{CN})_6]^{3-}$. The coatings were applied to glass slides, cured, and submerged in Sea water at $20 \pm 2^\circ\text{C}$ at 30 rpm in brown Duran bottles. Samples of the lixiviates were taken after 1, 3, 7, 10 and 30 days and tested on the marine microalgae *Tetraselmis chuii* and the crustacean *Artemia salina*. Cultures of *T. chuii* were maintained at $20 \pm 1^\circ\text{C}$ and a photoperiod of 12 h light: 12h dark in medium (F/2+ Si Guillard). Growth inhibition following exposure tests was evaluated following the ISO 10253:2016 adapted to 24 wells microplates using raw lixiviates or after filtration at $0.45\ \mu\text{m}$. *A. salina* nauplii were transferred to lixiviates and incubated at $25 \pm 2^\circ\text{C}$ for 24 hours when their mobility was assessed. For microalgae assays, filtration had no impact on the toxicity of coatings with LDH but increased the toxicity of coatings with Fe_3O_4 . Lixiviates of all coatings LAPOSS 1 induced around 50% microalgae growth inhibition regardless of the day of sampling. Lixiviates of LAPOSS 1 with Fe_3O_4 or LDH at 5 wt% induced similar growth inhibition levels compared to the blank coating, indicating the toxic effects observed originated from compounds lixiviated from the coating matrix of LAPOSS 1, and these components were already lixiviated 24 hours after immersion. Lixiviates from the coating LAPOSS 3 did not induce marine microalgae growth inhibition. Immobility tests with *A. salina* results indicated the lixiviates of LAPOSS 1 and 3 with or without additives had no toxic effects to the crustacean even after 30 days of lixiviation.

6.03.P-Th201 Bioaccumulation of Non-Dissolvable Engineered Nanomaterials in Freshwater Aquatic Organisms: A Meta-Analysis of the Available Data

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Understanding the bioaccumulation of engineered nanomaterials (ENMs) is essential for regulatory decisions on potential environmental risks. Research in the field of ENMs bioaccumulation has increased in recent years but the compilation and statistical analysis of the available experimental data has not been updated. We therefore performed a meta-analysis of the existing laboratory and field data on the bioaccumulation of eight types of non-dissolvable ENMs (TiO_2 , Al_2O_3 , Au, C_{60} , fullerenes, carbon nanotubes, FeO_x , graphene, polystyrene) in non-mammalian freshwater aquatic organisms across three trophic levels including phytoplankton, zooplankton and fish. Three typical endpoints were used to assess the bioaccumulation potential: the bioconcentration factor (BCF), the bioaccumulation factor (BAF) and the biomagnification factor (BMF). Our results suggest that zooplankton has greater mean logarithmic BCF and BAF values than phytoplankton (3.31 vs. 1.42) and fish (2.04). ENMs are biomagnified in zooplankton with a mean BMF of 17.4 whereas trophic transfer from primary consumers (zooplankton) to secondary consumers (fish) is not observed (mean BMF of 0.13). No clear dependency was identified between the physico-chemical characteristics of ENMs (e.g. primary particle size, hydrodynamic diameter or zeta potential) and bioaccumulation, except for coated vs. uncoated particles in phytoplankton. Carbonaceous ENMs were found to be more bioaccumulated than the other ENMs, except TiO_2 . The meta-analysis of bioaccumulation data can i) deepen the understanding of bioconcentration, bioaccumulation and biomagnification of ENMs, ii) be used to support grouping strategies as a basis for a safer-by-design approach for ENMs, iii) be integrated into comprehensive hazard and risk assessments, iv) promote the standardization of testing guidelines, and v) enhance the future kinetic bioaccumulation modelling.

6.03.P-Th202 The Bioaccumulation Testing Strategy for Nanomaterials: Physico-Chemical Triggers and Read Across From Earthworms in a Meta-Analysis

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Determining the bioaccumulation potential of manufactured nanomaterials (MNs) is a key part of environmental risk assessment for chemicals. For traditional chemicals, the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 305, bioaccumulation in fish is often used. However, for MNs, there are no approved processes to trigger or waive this test, or consider alternatives to vertebrate animals. A meta-analysis of existing data sets on particle properties and bioaccumulation in earthworms was undertaken in order to understand what particle metrics could be used as a trigger for bioaccumulation testing. An apparent steady state tissue concentration of metal from MNs exposure in the earthworm (*Eisenia fetida*) was evident following exposures to Ag nanoparticles (NPs), CuO NPs and CdTe quantum dots (QDs). Nano bioaccumulation factors ($n\text{BAFs}$) were calculated using soil and earthworm tissue metal concentrations. A prediction equation using all the particle metrics correlated with BAFs was possible. Similarly, nano biomagnification factors ($n\text{BMFs}$) were calculated in the rainbow trout

(*Oncorhynchus mykiss*) tissue, relative to the concentration of total metals in the fish diet. Pearson's correlations were found to be significant, with $p < 0.05$ for n BMFs for the liver, mid intestine, hind intestine and kidney relative to the earthworm tissue n BAFs. The results with silver and copper nanomaterials showed that the earthworm bioaccumulation test is predictive of the fish bioaccumulation test and that there is scope to predict the bioaccumulation potential of MNs with confidence from a few simple particle metrics.

6.03.P-Th203 The Bioaccumulation Testing Strategy for Nanomaterials: Correlations With Particle Properties and a Meta-Analysis of In Vitro Alternatives to In Vivo Fish Tests

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In a regulatory context, determining the potential of substances to bioaccumulate is a key part of the chemical safety assessment. For manufactured nanomaterials (MNs), given the breadth of forms produced, it is not ethical or practical to test all materials using vertebrates in line with the 3R's (replacement, reduction, refinement). This study aimed to show how alternative methods could predict the *in vivo* bioaccumulation potential of MNs in fish. This included exploring the physico-chemical properties of MNs as predictors of bioaccumulation, using the *ex vivo* gut sac technique to measure total metal uptake, and an *in chemico* digestibility assay to simulate the bioaccessible metal in the gut lumen of fish. An apparent plateau in net metal accumulation by rainbow trout was evident from data on dietary exposures for 4 weeks to CuO nanoparticles (NPs), Ag NPs or Ag₂S NPs *in vivo*. From the metal concentrations in the tissues compared to the diet, it was possible to derive nano biomagnification factors (n BMFs) from tissue total metal concentrations approaching steady state, which varied between organs and materials from 0.0004 to 1.5162. For instance, the CuSO₄, CuO NPs, AgNO₃, Ag NPs and Ag₂S NP liver BMFs were 0.3782, 0.3942, 1.3186, 1.3372 and 0.1240, respectively. The n BMF for the liver showed the best correlations with the physico-chemical parameters, with a significant correlation to the particle dissolution rate (Spearman's correlation, $p < 0.01$). The mid intestine mucosa of the *ex vivo* gut sac technique also gave good correlations to *in vivo* liver tissue total metal concentrations, with r^2 values between 0.8 and 0.9. For example, the correlation coefficients between the mid intestine mucosa and the liver tissue total metal concentrations of the CuSO₄, CuO NPs, AgNO₃, Ag NPs and Ag₂S NPs were 0.9067, 0.7639, 0.9167, 0.8326 and 0.9142, respectively. Moreover, there was a significant relationship between the total metal released in the stomach compartment of the digestibility assay and the total metal concentration in the liver of trout *in vivo* (Pearson's correlation coefficient, $p = 0.02$), suggesting the *in chemico* digestibility assay can predict the bioaccumulation potential of the ENMs. In conclusion, the meta-analyses supports the development of an integrated and tiered approach to bioaccumulation testing that considers the 3Rs and minimises the use of the fish bioaccumulation test (e.g., OECD TG 305), for manufactured nanomaterials.

6.03.P-Th204 Environmental Fate of CeO₂ Nanoparticles - Critical Evaluation of Available Data and Implications for Exposure Assessment

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Cerium dioxide nanoparticles are being used in important applications such as automotive catalysts, industrial polishing, and various niche applications. As of 1 January 2020, REACH dossiers needed to be updated taking into account the various information requirements for nanomaterials as stipulated by the amended REACH Annexes, although for multiple endpoints no harmonised or internationally standardised methodologies dedicated to the testing of nanomaterials were available yet. For cerium dioxide, a huge amount of information has been published or made publicly available over – predominantly – the past two decades. Inevitably, studies were performed according to different methodologies, which complicates their overall assessment. In view of the update of the REACH dossier, this huge amount of information has been critically evaluated, taking into account current recommendations for the environmental fate testing of nanomaterials (where available), to arrive to a selection of sources that – in a weight of evidence approach – best reflects the findings on the different endpoints. In this poster, an overview will be given of the approach followed and the main findings. Additional discussion will be included on the implications for environmental exposure assessment and the assessment of exposure of humans via the environment, the mismatch between input parameters for available exposure models and available data, and the possibilities to assess exposure and risk in a qualitative or semi-quantitative way.

6.03.P-Th205 Marine Hazard Assessment of Soluble and Nanostructured Forms of the Booster Biocide DCOIT in Tropical Waters

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Recently, the encapsulation of commercial AF biocides, such as DCOIT, in mesoporous silica nanocapsules (SiNC) has demonstrated to be an efficient and eco-friendly alternative to current state-of-the-art booster biocides. However, there is little information on the chronic effects on non-target tropical species, which is critical for a more comprehensive environmental risk

assessment of such innovative nanomaterials. Thus, the present study aimed at assessing the chronic toxicity of both soluble and encapsulated forms of DCOIT (DCOIT and SiNC-DCOIT) on tropical marine species as well as at assessing the chronic hazard of both conventional and nanostructured AF additives in the tropical environment. To accomplish this goal, short and long-term chronic exposure tests were carried out on six ecologically-relevant tropical marine species, including bacteria, fungi, microalgae, and sensitive life stages of crustaceans and echinoderms, for which NOEC values were calculated. For the marine hazard assessment, NOEC values available in the literature for other marine species reported on tropical waters were also considered. The marine tropical hazard assessment of DCOIT and SiNC-DCOIT was based on the determination of the predicted no-effect concentration ($PNEC_{\text{marine}}$) using the probabilistic approach (species sensitivity distribution method). As the main findings, DCOIT was the most toxic compound for all tested tropical species (lowest $NOEC=0.03 \mu\text{g DCOIT/L}$). Apart from the tested bacteria and fungi ($NOEC \geq 100 \mu\text{g/L}$), the nanostructured form of DCOIT was 3 to 10-fold less toxic than its free soluble form. Probabilistic-based $PNEC_{\text{marine}}$ was set on 0.0001 and 0.0097 $\mu\text{g DCOIT/L}$ for the soluble and nanostructured forms of DCOIT, respectively. Accordingly, the immobilization of DCOIT into SiNC led to an 84-fold hazard decrease which is more expressive compared with the most conservative approach to determine the $PNEC$ values for temperate species (2-fold decrease). These findings confirmed that the encapsulation of DCOIT into SiNC is a promising eco-friendly alternative technique, even in a chronic exposure scenario. Finally, the present study brought contributions of paramount importance to better understand and manage the environmental risks of such innovative products in marine tropical areas.

6.03.P-Th206 Form-Specific Environmental Risk Assessment of Three Engineered Nanomaterials (Nano-Ag, Nano-TiO₂, and Nano-ZnO) in Freshwaters

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Engineered nanomaterials (ENMs) are widely applied in many commercial products and applications. The fate and toxicity of ENMs vary depending on the forms in which the ENMs are released into the environment (i.e. pristine, dissolved, transformed, and matrix-embedded forms). So far, environmental risk assessments for ENM have not considered the released form. The aim of the current study was to estimate the form-specific environmental risk of nano-Ag, nano-TiO₂ and nano-ZnO for European freshwaters. The form-specific material flow analysis was based on Adam et al., (2018). Probability distributions of predicted environmental concentrations (PEC) for freshwaters were obtained for the different forms. Probabilistic species sensitivity distributions were obtained for the different released forms based on an analysis of the ecotoxicological literature. The risk characterization ratio (RCR) was calculated by dividing the PEC distributions by the $PNEC$ distributions. The total risk of an ENM was determined by summing the RCRs for each ENM form.

All RCR distributions of the different forms of nano-Ag and nano-TiO₂ were lower than 1 and indicated acceptable environmental risks. None of the means of the RCR distributions of different forms of nano-ZnO were greater than 1. However, 20% of the RCR estimations for pristine ZnO and 1.45% of the RCR estimations for transformed ZnO exceeded the value of 1. The overall risk of ENM considering the released forms was compared with the standard approach which computes RCR values regardless of the released form and is based on the hazard assessment of the pristine nanoparticulate form. The overall risk of nano-Ag and nano-ZnO decreased by considering the released form. The RCRs of nano-TiO₂ were estimated to be the same in both methods as transformation or dissolution is not relevant for this material.

The results of this work show that there is now sufficient information available to perform form-specific risk assessments for ENM. The first results indicate that considering the released form changes the risk assessment and thus a further extension of this approach and incorporation into regulatory risk assessment needs to be moved forward.

6.03.P-Th207 Environmental Hazard Assessment of CeO₂ Nanoparticles - How to Evaluate the Huge Amount of Available Ecotoxicological Data in a Transparent Way for Regulatory Purposes

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Cerium dioxide nanoparticles are being used in important applications such as automotive catalysts, industrial polishing, and various niche applications. As of 1 January 2020, REACH dossiers needed to be updated taking into account the various information requirements for nanomaterials as stipulated by the amended REACH Annexes, although for multiple endpoints no harmonised or internationally standardised methodologies dedicated to the testing of nanomaterials were available yet. For cerium dioxide, a huge amount of information has been published or made publicly available over – predominantly – the past two decades. Inevitably, studies were performed according to different methodologies, which consequently resulted in contrasting findings. In view of the update of the REACH dossier, this huge amount of information has been critically evaluated, taking into account current recommendations for the testing of nanomaterials, to arrive to a selection of sources that – in a weight of evidence approach – best reflects the findings on the different ecotoxicological endpoints. In this poster, an overview will be given of the approach followed and the main findings. Where applicable, additional discussion will be dedicated to the impact of methodology on the observations in the different studies, and to the importance of studying sub-apical endpoints to unravel mode of action as well as apical endpoints to retain a view on the potential ecological relevance of observed effects, if any. Also, the attention is drawn to the fact that for risk assessment purposes, there is a clear need to fully understand the environmental fate and behaviour of the nanoparticles – discussed in the poster “Environmental fate of CeO₂ nanoparticles – critical evaluation of available data and implications for exposure assessment” also submitted in this Session – to be able to place any observed effects into perspective.

6.04 Impact assessment of chemical alternatives and beneficial substitution

6.04.T-01 Delphi Study to Identify and Assess Factors Specifying the Concern of Persistent Chemicals to Support Risk Management

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Due to the lack of information on risks or impacts on the environment and on human health, a fundamental challenge for effective risk management of PBT/vPvB substances is therefore, to prioritise those substances for regulatory action which are of highest concern. So far, there is no comprehensive and systematic approach identifying which information on PBT/vPvB substances is (most) relevant in order to support risk management decisions. This also applies to new hazard categories that are currently under debate such as PMT substances. Obviously, expert knowledge is an important source of information to support prioritisation and decision-making in the risk management of PBT/vPvB substances. To date, a thorough understanding of what experts would consider to be relevant factors characterising the concern of persistent chemicals is lacking. The purpose of this study is to explore experts' opinions on what factors they consider relevant for specifying the concern of PBT/vPvB substances to risk managers. Specifically, the purpose of the study is to contribute to a better understanding of the PBT/vPvB concern by exploring · what factors experts consider to be important to specify the concern about PBT/vPvB substances. · how experts evaluate the importance of these factors. · the level of agreement among experts. · if there are differences in the opinion of experts from different groups (i.e. science, authorities, industry and NGOs) or different global regions. In our talk we will follow up on the presentation during SETAC Europe 2021 by providing insights into the process, the intermediary results as well as the lessons learnt of this Delphi study. The results of this Delphi study can facilitate a better understanding of the PBT/vPvB concern, which contributes to a better-informed risk management of persistent chemicals in different ways: (i) by exploring factors considered to characterise this concern, (ii) by revealing level of agreement among experts and potential patterns between different stakeholder groups and (iii) by providing input to prioritising information to support regulatory strategies and decision-making on phase-out of these chemicals, as envisaged under, for example, the European Chemicals Strategy for Sustainability.

6.04.T-02 Applying the Essential Use Concept Within the REACH Authorisation Process

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Since the suggestion to implement the essential use concept (EUC) from the Montreal Protocol to determine when uses of PFAS should be phased out the concept has been discussed in the peer-reviewed literature as well as regulatory settings such as the new EU Chemicals Strategy for Sustainability (CSS). Researchers developed the categories “non-essential”, “substitutable” and “essential” to evaluate necessity of the continued use of a substance of concern. At present, it is not clear if and how this categorisation approach can be integrated into existent chemicals regulation, i.e. the REACH Authorisation process, in order to decide if a continued use is essential or not. Further, if a substance is anticipated to be substituted, there is a risk that the alternative is not safer than its predecessor (known as regrettable substitution). Thus, to reach the goal of a non-toxic environment as stated in the CSS the assessment of alternatives needs to be done in a comprehensive, thorough, and transparent way. The objectives of this study were to (a) investigate if and how the EUC has been (implicitly) referred to in the REACH Authorisation process, (b) analyse how alternatives to substitutable substances are assessed in applications for authorisation at present, and (c) clarify and suggest how the EUC could fit in the REACH Authorisation process. We analyse REACH regulation articles 55 to 66 and the applications for authorisation by industry on which the ECHA's Committee for Socio-Economic Analysis (SEAC) has published its final opinion on. Elements of the EUC are implicitly incorporated in the REACH Authorisation process as companies are required to justify the importance of the function provided by a SVHC in a particular use in order to get an authorisation. They are also supposed to show the socio-economic impacts if they were not able to use the substance anymore. There is still a need for guidance in order to effectively integrate and apply the EUC for REACH Authorisations. This could be a questionnaire that results in a “degree of essentiality”, and includes a statement of values and preferences. Preliminary results further suggest that the hazard screening of alternatives performed by applicants for authorisation lack of thorough evaluation of (eco-) toxicological endpoints, data and transparency which hampers identification of safer alternatives and could result in regrettable substitution.

6.04.T-03 Persistent but Pivotal - Which Uses of Persistent Chemicals DO European Citizens Consider Essential or Non-Essential for Society?

Annika Karinen¹, Silke Gabbert², Jeroen Devilee¹, Arianne de Blaeij³, Hilde Tobí⁴ and Martijn Beekman², (1)Dutch National Institute for Public Health and the Environment (RIVM), Netherlands, (2)National Institute for Public Health and the Environment (RIVM), Netherlands, (3)National Institute for Public Health and the Environment RIVM, Netherlands, (4)Wageningen University, Netherlands

One aim of the European Chemicals Strategy for Sustainability (CSS) is to phase out Substances of Very High Concern (SVHC), such as chemicals classified as persistent, toxic, and bioaccumulative (PBT) or very persistent and very bioaccumulative (vPvB), unless their use is considered essential for society. However, clear criteria to distinguish between essential and non-essential uses are yet to be identified. Hence, the aim of this project is threefold. First, we provide an overview of the criteria for essential use that have been suggested in the literature. This literature review illuminates different criteria and frameworks to define essential use, as well as insights into past applications of the essential use criteria as specified in the Montreal Protocol, a multinational environmental agreement to phase out ozone-depleting substances. Second, we present the results of a participatory stakeholder consultation aimed at identifying societal goals that the use of persistent chemicals should contribute to in order to be considered essential for society. In total, 14 societal goals emerged: health, food and water, shelter, energy, safety, security, climate and the environment, mobility, communication, education, culture, recreation, innovation, and economy. These results suggest

considerable diversity in stakeholder views considering essential use of persistent chemicals. Third, we present the first results from an EU citizen's survey examining which uses of persistent chemicals do EU citizens consider essential or non-essential for society. The EU citizen's survey is, to the best of our knowledge, the first to examine citizen's perspectives on essential use of persistent chemicals. The results from this survey will inform us of the uses that EU citizens consider essential or non-essential for society, whether emphasizing the consequences of allowing or not allowing the use of persistent chemicals affects essentiality ratings, and whether nationality, age, gender, socioeconomic status, education, or political orientation affect essentiality ratings. Data collection will take place in early 2022, and the first results from this survey will be presented at the SETAC plenary.

6.04.T-04 Comparative Ranking of the Long-Term Impact Potential of Chemical Substances

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Replacing substances of concern by less harmful alternatives is a straightforward strategy for reducing the environmental and health impact of chemicals. A new concern-based scoring scheme allows for flexible and transparent comparative ranking of the impact potential of chemical alternatives (SCoRA). The approach accounts for hazards due to ecotoxicity to organisms in water/sediment and soil, and effects on human health such as CMR properties and endocrine disruption. Exposure-related information, in particular the expected environmental pollution stock levels, is then also taken into account. Both hazard and exposure information are translated into ordinal scores and weighted with factors addressing substance- and scenario-related uncertainties due to (yet unknown) long-term impacts. Aggregation of the individual substance-specific scores yields the overall impact potential, which is used for an ordinal ranking of chemicals. Visualisation of concern components by a heatmap and fingerprints allows for a practical comparison of the potential impact of different substances. The SCoRA approach is illustrated using case study chemicals of very high concern (15 SVHC, mostly PBT, representing different chemical classes with different modes of bioaccumulation and toxicity). Notably, there are substantial differences between the impact profiles of these substances. CMR properties stand out in addition to PBTness. Fingerprinting underlines that, by covering a broad range of concerns, SCoRA goes well beyond binary screening criteria, e.g. for PBTness, and allows for a comprehensive characterisation of concerns especially in cases where a particular metric is just below, close to or just above a threshold. SCoRA, therefore, offers a complement to established chemical assessment tools under REACH and other regulations. In conclusion, SCoRA is an effective tool to compare and to communicate the impact potential of chemicals based on consistent criteria (impact scores) and supports decision-making towards safe and sustainable substitution.

6.04.T-05 Moderated Discussions

Monika Nendza, Analytisches Laboratorium, Germany
Moderated Discussions

6.04 Impact assessment of chemical alternatives and beneficial substitution (Poster)

6.04.P-Th208 Ecotoxicological Assessment of Two Potential Environmentally Friendly Red Dyes

Natalia de Farias¹, Anjaina Fernandes de Albuquerque², Harold S. Freeman³, Riikka Raisanen⁴ and Gisela Umbuzeiro⁵, (1)UNICAMP, Limeira, Brazil, (2)UNICAMP - University of Campinas, Brazil, (3)North Carolina State University, United States, (4)University of Helsinki, Finland, (5)School of Technology- UNICAMP, Brazil

Research on natural resources has increased and, biocolourants have been investigated as an alternative source of color for textiles to synthetic dyes, which can have harmful effects on the environment and ecological balance. The possibility of using natural dyes to help achieve the production of sustainable materials has not been properly explored. The objective of this work was to evaluate the aquatic toxicity of two highly purified anthraquinone red dyes, dermocybin (>99%) and dermorubin (>98%) obtained from the fungi *Cortinarius sanguineus*. Tests were conducted in concentration-response experiments. Dyes were dissolved in Dimethyl sulfoxide (DMSO) at the limit of solubility and tests were conducted with a maximum of 0.01% DMSO as recommended by OECD 23. A chronic toxicity test was performed with the freshwater *Pseudokirchneriella subcapitata* according to OECD guideline 201. After 72h of exposure to dermocybin (0.1 – 0.7 mg/L) and dermorubin (0.0625 – 1 mg/L), growth inhibition was evaluated and the IC₅₀ was determined. Acute toxicity tests with *Daphnia similis* were performed according to OECD guideline 202. Immobilized organisms were counted after 48h of exposure to dermocybin (0.035 – 0.7 mg/L) and dermorubin (0.03 – 1 mg/L), and EC₅₀ was determined. For lethal and sub-lethal toxicity assessment a fish embryotoxicity test (FET) was performed according to OECD guideline 236 with the extension of time exposure of 96h to 168h at concentrations range 0.01 to 1 mg/L for dermorubin. Mortality and developmental abnormalities were registered daily. FET test with dermocybin is underway. For both dyes, it was not observed significant differences in cell density of microalgae between treatments and the control group, with an IC₅₀ > 0.7 and 1 mg/L for dermocybin and dermorubin, respectively. Dermorubin was not toxic to *D. similis* with an EC₅₀ > 1 mg/L, while dermocybin showed acute toxicity, with an EC₅₀ of 0.99 mg/L. Dermorubin did not affect the survival of embryos/larvae or caused any other sublethal effect, EC₅₀ > 1mg/L. Our findings emphasize that those two dyes seem to be promising and can be part of a family of environmentally friendly natural dyes for traditional and waterless dyeing technologies. For future studies, chronic tests will be performed with *Ceriodaphnia dubia* and also, we will evaluate the genotoxicity of the dyes using the Comet assay with zebrafish.

6.04.P-Th209 Risk Indicators for Pesticides Based on Sales, Exposure, Ecotoxicity and Mitigation

Muris Korkaric¹, Lolita Ammann¹, Irene Hanke¹, Jérôme Schneuwly¹, Mikko Lehto¹, Thomas Poiger¹, Laura De Baan¹, Otto

Daniel¹ and Judith F. Blom², (1)Agroscope, Switzerland, (2)Plant Protection Products - Impact and Assessment, Agroscope, Switzerland

The European Commission's «Sustainable Use of Pesticides» Directive aims to reduce the use of pesticides and the associated risks by promoting alternative approaches or lower-risk techniques. To measure progress towards the main objective of this Directive, the «Harmonized Risk Indicator» (HRI) has been introduced. It shows the change in sales volumes of active ingredients of plant protection products (PPPs) divided in four categories, with each category assigned a weighting that is used when adding up the sales volumes. However, as the HRI does not consider exposure (i.e. the concentration of active ingredients in environmental compartments) or ecotoxicity, risk mitigation measures cannot be accounted for. The aim of this study was to develop suitable risk indicators for PPPs that can show trends in the risks to organisms in surface waters and terrestrial habitats, as well as groundwater pollution. These indicators are based on the sales figures of PPP active ingredients and take into account their exposure and ecotoxicity as well as the effects of risk mitigation measures. To calculate the risk indicators, a *Treated Area* is determined for each active substance and multiplied by a *Risk Score* and a *Risk Reduction* factor. The *Treated Area* is estimated by dividing the annual sales figures of each active ingredient by its average agricultural application rate. The *Risk Score* expresses the exposure of the active ingredients in each environmental compartment based on a standardized application, taking into account the environmental chemical properties of the active ingredients. For the risk indicators «Surface Water» and «Terrestrial Habitats», the exposure is multiplied by the ecotoxicity for aquatic and terrestrial organisms, respectively. The *Risk Reduction* factor considers product-specific mitigation measures as well as general risk-reducing measures (e.g. in relation to drift or run-off) and their rate of implementation. The new approach presented here allows to show both changes in sales of high-risk PPPs and effects of product-specific and general mitigation measures. This is a clear advantage over existing risk indicators such as the HRI and can help to assess the risk-reduction goals of national policies. Moreover, the calculation on a factor-by-factor basis also provides sufficient flexibility to incorporate future mitigation measures as well as new data on implementation rates.

6.04.P-Th210 Bioadditives for Sustainable Energy Production

Ogemdi Chinwendu Anika, MSc and Raffaella Villa, De Montfort University, United Kingdom

Imagine what goes on in the stomach of cows when they feed on grass and what comes out as by-products. Also imagine the possibility of trapping the gaseous components of the by-products in a balloon for use as an energy source. All the activities that occur in the stomach of cows as they feed are termed anaerobic digestion, which is the breakdown of organic matter in the absence of oxygen to release biogas rich in methane and solid deposits that can serve as organic fertiliser. The problem, however, is that agricultural emissions, particularly that from the stomach of cows are major contributors to greenhouse gases in the environment. It is, therefore, necessary to cut down on these emissions, manage agricultural wastes and generate valuable energy by mimicking the activities that occur in the stomach of cows in enclosed systems such as bioreactors. The challenge, still, is that agricultural wastes are composed of materials that are too difficult to degrade and bioreactors are not sophisticated like the stomach of cows that are highly efficient at breaking them down. One way of resolving this challenge is by treatment with bioadditives, which are cheap, naturally sourced and environmentally friendly. Bioadditives are already used in industry with positive results but are still not optimized because there is still a gap in the knowledge of how they work. Consequently, the aim of this research, is to understand their mechanisms of action to optimize the system. So, by manipulating bioadditives in lab scale bioreactors, this research is positioned to discover how bioadditives can be used correctly to improve the anaerobic digestion process, sustainably produce more energy and stable organic fertilizer for the green farmer.

6.04.P-Th211 Importance of Customising Bioadditives Dose for AD Optimisation

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Anaerobic digestion (AD) has been described as the golden technology for alleviating environmental issues associated with waste generation from the agricultural sector as well as representing a sustainable waste biorefinery from cheaply sourced agricultural waste biomass. The major component of agricultural biomass, lignocellulose, is composed of different polymers: cellulose (15–99%), hemicellulose (0–85%) and lignin (0–40%). These polymers have different biodegradability levels in anaerobic conditions and their ratios in the agricultural waste will affect the degradation process in different ways. This study aimed to evaluate the response of two different lignocellulosic waste biomass (ensiled corn and rye) to a commercial yeast-based bioadditive. The results showed that rye was more responsive to the bioadditive than corn as the bioconversion time was shortened by half while improving the biogas and methane yields when compared to the control by 58% and 100% respectively with a 2mg addition. Biogas increase for corn was limited to 21% with the 10 mg dose although the quality of the biogas was higher for all doses and for both substrates: 35% in corn control samples vs 37%, 41% and 38% for 1, 2 and 10 mg bioadditions respectively with corn and 47% in rye control samples vs 58%, 61% and 47% for 1, 2 and 10 mg bioadditions respectively with rye.

6.04.P-Th212 Proactive Environmental Hazard Assessment of Liquid Organic Hydrogen Carriers (LOHCs) As Novel Carriers of Renewable Energies

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The development of renewable energy is an indispensable step toward a sustainable society. Liquid Organic Hydrogen Carriers (LOHCs) are very promising vectors for hydrogen storage and transportation. LOHC systems consist of a pair of H₂-lean, typically (poly)cyclic aromatic compounds, and H₂-rich form, (poly)cyclic heteroaromatic compounds. The scale of LOHC implementation can reach that of liquid fossil fuels if full replacement shall occur – in this scenario there is a high likelihood of release of LOHC chemicals into the environment. Until now, the LOHC systems based on dibenzyltoluene and benzyltoluene are

technically mature but there are some technological hurdles. Thus, it is of high interest for LOHC developers to find potential LOHC, which allow similar hydrogen capacity but also similar or better hazard profile. To avoid regrettable substitution (of fossil fuels or one LOHC system by another) a comprehensive hazard profile is crucial before widespread use. This study aims to understand the harmful effects of novel LOHC compounds by conducting ecotoxicity tests with *Raphidocelis subcapitata*, *Daphnia magna* and *Vibrio fischeri*, and biodegradation test to determine their persistence. Additionally, the octanol-water partition coefficient ($\log K_{ow}$) is used to build Quantitative Structure Activity Relationship (QSAR) to determine the mode of toxic action of novel LOHC compounds. The exposure concentration of test solutions in ecotoxicity tests was measured using LC/MS-MS. The comparative hazard assessment was conducted including comparisons between different forms of the same carrier (H_2 -lean vs H_2 -rich forms) and between LOHCs and conventional energy sources (fossil fuels). All tested LOHC compounds seem to be baseline toxicants (i.e. toxicity is driven by hydrophobicity of compounds). H_2 -lean form of novel LOHC is readily biodegradable. On the other hand, H_2 -rich and partially hydrogenated forms are rather less biodegradable. Overall, tested LOHC compounds seem to have a similar level of environmental hazard to that of diesel oil based system. The research outcome will be of the highest interest to environmental scientists, LOHC developers (in academia and industry), regulatory agencies, governments, and the general public.

6.04.P-Th308 Toxicity Testing of Technical Mixtures of Liquid Organic Hydrogen Carriers

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Hydrogen has the potential to become a carbon neutral energy source in the upcoming century, especially if it is produced using excess renewable energy. However, currently we lack efficient storage methods, as hydrogen itself suffers from very low volumetric energy density and (cryo)compressing requires specialised infrastructure, is costly and rises issues in terms of handling safety. In recent years a new possibility of hydrogen storage emerged with liquid organic hydrogen carriers (LOHC) whereby hydrogen is chemically bound to an unsaturated, mostly aromatic, compound. Through a catalytic process the hydrogen is then recovered and can then be used (e.g., in fuel cell). Substances like (di)benzyltoluene, carbazoles, benzenes, etc. are currently being investigated or entered the market. However, little is known about the environmental behaviour of these compounds. Their physical-chemical properties (mainly relatively high hydrophobicity) suggest considerable toxicity and bioaccumulation potential. Reliable toxicity tests require a constant exposure concentration to provide solid results. To achieve that and mitigate losses during the tests (e.g., adsorption to organic matter or vessel walls, evaporation) passive dosing via a PDMS reservoir is used during the experiments. By controlling and varying the loading of the PDMS reservoir with the test substance it is furthermore possible to adjust the concentration in the test medium. Thus, it is possible to not only conduct limit tests (at the maximum water concentration) but with this approach a full dose-response tests are possible. We could show that there is a linear relationship between the loading of the polymer (up to 10 % in methanolic solution) with benzyltoluene and the resulting concentration in the medium. We furthermore conducted acute algae and daphnia toxicity tests of technical grade mixtures of LOHC based on benzo- and dibenzotoluene. We showed acute toxicity for a benzyltoluene isomer mixture and furthermore determined an EC_{50} value for that compound.

6.05 Mixtures matter – from science to regulation and management

6.05.T-01 How to Operationalize the Mixture Assessment Factor (MAF) for Facilitating the Regulatory Risk Assessment of Chemical Mixtures?

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The new EU Chemicals Strategy for Sustainability (CSS) states that “The Commission will: assess how to best introduce in REACH (a) mixture assessment factor(s) for the chemical safety assessment of substances;” Furthermore, the corresponding Annex states that the Commission will assess until 2022 how to best introduce such a MAF (mixture assessment factor) into Annex I of REACH. However, details on the operationalization of a MAF are largely missing. This presentation will first provide a brief overview of the recent regulatory developments in European chemical policy, especially exploring the conceptual demands on a MAF in the context of the CSS and the EU’s Zero Pollution Action Plan. It will then present and compare different algorithms on how to estimate an adequate size of the MAF for a given mixture, i.e. a MAF size that is sufficiently protective, but not excessive. All algorithms are based on the assumption that the mixture behaves largely concentration-additive and acknowledge the typical Pareto-distribution of individual toxic units (risk quotients). It determines the maximum tolerable total concentration of the mixture, i.e. the PNEC(mixture) using a level of protection similar to the individual chemicals. The outlined approaches will be applied to a range of published case studies, including chemical monitoring data from various chemical monitoring programs, with a focus on mixtures comprising pesticides, pharmaceuticals and industrial chemicals. Results indicate that a MAF of 5 to 50 is sufficiently protective, dependent on the chemical complexity of the mixture. Between roughly 10% and 30% of the mixture components are affected by the application of a MAF, i.e. their concentrations exceed the critical value of PNEC/MAF.

6.05.T-02 In Depth Characterisation of Chemical Co-Exposure and Cumulative Risks of Environmental Monitoring Data in the EU

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Chemicals are typically regulated on a chemical-by-chemical basis in which regulatory frameworks set safe exposure levels. As a result, current environmental risk assessment procedures of chemicals focus on exposure to individual chemicals. However, the

recognition is growing that exposure to a cocktail of chemicals may cause risks not captured by these single chemical evaluations. Monitoring datasets offer a unique perspective on this issue as they can provide information on the co-exposure patterns of chemicals. In the current analysis the focus was on three databases: EU Waterbase, Rhine (ICPR) and EauFrance. To understand which mixtures occur in the environment, principal component analysis (PCA) was used as a data mining technique to identify and characterize observed mixtures. Additionally, cumulative mixture risk was analyzed using different techniques. The majority of the chemicals are not detected (50-80% of chemicals) and a limited set of chemicals is nearly always included in the monitoring strategies (mainly priority pollutants). The PCA revealed that most of the mixtures in the databases consist of a wide range of chemicals and are not determined by specific chemical groups. Cumulative risk analysis showed that the vast majority of the mixtures are not contributing to risk: 84-94% of the samples were predicted as being of no concern, even with a conservative assumption of additivity. The percentage of mixtures with potential concern not identified by single substance assessment is small (

6.05.T-03 MAF Implementation for Inorganic Substances: REACH Impact Assessment and Identification of Inorganic Priority Contributing Substances I-Pcs

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Policy makers are moving forward with the combination effects assessment of non-intentional mixtures, by proposing a Mixture Assessment Factor (MAF) under REACH. A preliminary analysis of REACH registered chemicals, performed by ECHA, indicated that a blanket MAF of 10 would allow to focus on the most relevant substances. However, inorganic substances were not included in this assessment. For these substances it is expected that such a generic MAF would be problematic for natural occurring substances. Therefore, the objectives of this study were the following: (i) assess the impact of a generic MAF on the risk characterisation ratios (RCRs) in REACH dossiers of inorganic substances; and (ii) identify ten inorganic-priority contributing substances (I-PCS) based on monitoring data for further follow-up research. The first part of the study was based on RCRs taken from the exposure scenarios in the Chemical Safety Reports (CSR) of the REACH dossiers. In total 16 inorganics were included with 846 contributing exposure scenarios out of 51 CSRs. A MAF of 10 would mean that 76% of the contributing exposure scenarios for freshwater and soil would need further refinements. A MAF of 10 applied to reasonable worst-case regional background values for freshwater will already result in RCRs > 1 for 8 out of the 16 inorganics included in this assessment. The issue is thus complicated for many inorganics by the relatively high regional background value compared to the PNEC. Scenarios to improve the reference RCRs seems only feasible for a limited number of data-poor metals. In the second part of the study, three monitoring databases were considered to identify the I-PCS: two freshwater databases (Waterbase, FOREGS) and one soil database (GEMAS). Risk quotients were calculated for all inorganics based on the measured concentrations divided by effect concentrations (both PNEC and HC5 values were evaluated). The inorganic substances most contributing to environmental risk across monitoring databases were (in alphabetic order) Ag, As, Cd, Co, Cr, Cu, Hg, Li, Mn, Ni, Pb and Zn. The choice of the effect value was critical for most inorganics, i.e., the number of samples at risk was lower using the HC5 versus the PNEC. Refinements for individual inorganics are needed to reduce the uncertainty on calculated risks. Further research will be initiated to determine the actual environmental combined risks posed by these metals in a mixture context with other metals and organics.

6.05.T-04 Petroleum Refinery Effluent Contribution to Chemical Mixture Effects in the Environment

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Petroleum refinery effluents (PRE) are wastewaters mainly from industries associated with oil refining. Within Europe, PREs are regulated through local discharge permits and they receive significant treatment before emission. After treatment, PREs can still contain various hazardous pollutants (e.g. hydrocarbons, ammonia, heavy metals). Earlier work, including whole-effluent toxicity assessments, has shown that toxicity of PREs is often limited. However, the extent to which PREs contribute to mixture pressure in the receiving environment is unknown. Environmental risks of PREs depend on several factors including effluent compositions and receiving water conditions: exact risks may vary from case to case. Therefore, our study aimed to develop a holistic approach to assess the mixture pressure of PREs both before and after discharge, using the multi-substance potentially affected fraction of species (msPAF) as an indicator. Dissolved concentrations of total petroleum hydrocarbons (TPH) in PREs were determined based on TPH concentrations and hydrocarbon block compositions, using the PetroTox model. Species sensitivity distribution data (SSD) were compiled, and the target lipid model (TLM) was applied to compute toxicity values for TPH. Based on concentrations and SSDs, msPAF levels were calculated for more than 80 PREs at discharge points and downstream (diluted in the recipient using standard dilution factors). Computed msPAF levels were compared with msPAF background levels in European waters to assess the PRE contribution. Lastly, computed msPAF levels were compared with bioassays to assess the level of conservatism of the method. The average mixture pressure of PREs at discharge points was estimated to be 73.5% (msPAF-chronic) and 30.2% (msPAF-acute). Mixture pressure was reduced downstream, and the extent depended on the corresponding receiving environment (dilution factor). Hydrocarbons (mainly TPH) and inorganics (mainly ammonia) explained at least 85% of the mixture pressure. PREs were likely to contribute less than other sources to pollution, while the contribution varied between 0.03% to 620% of msPAF background levels across sites. Compared with available bioassays, our msPAF estimates were regarded conservative. With proper chemical characterisation of PREs, our method could be applied for risk assessment of any PRE of interest. Site and substance rankings would help identify hotspots and take effective targeted action to remediate potential risks.

6.05 Mixtures matter - from science to regulation and management (Virtual Only)

6.05.V-01 An Industrial Pollution Investigation Reinforced the One Health Concept

Frannie Nilsen, NC DEQ, United States

Emerging compounds (EC) present unique challenges to environmental scientists and regulators alike, who are both often required to chase the contamination rather than design comprehensive studies. The lack of chemical information and toxicological knowledge about ECs complicates communication between scientists, regulators, and the affected communities. An affected community in North Carolina, USA, inspired regulators to investigate an industrial polluter in new ways and devise a holistic investigation strategy through environmental science. The holistic strategy included sampling air emissions, atmospheric deposition, soil, sediment, groundwater, and drinking water; testing the effectiveness of filtration units to remove the pollutants from drinking water; conducting non-targeted analyses to identify additional unidentified ECs, aquatic and mammalian toxicology studies; and sharing the newly found information with the affected community. To date, this holistic approach as identified contaminated drinking water sources more than 17 miles away from the industrial facility, provided the data needed to enforce emissions control requirements at the facility, identified several previously unidentified ECs, and elucidated the need for cross-pollination between media-specific groups. The investigation is still in progress and has been a continual effort since 2017. This situation and the complex nature of EC investigations have highlighted the One Health concept in a regulatory and enforcement setting as well as demonstrated the importance of diverse skillsets, backgrounds, and experiences in solving complex environmental problems.

6.05.V-02 Can Commercial Formulations of Pesticides Be Optimised Towards Environmentally Safer Alternatives?

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The production of environmentally safe commercial pesticide products is a current concern that has been managed by the development of very specific regulation. While the formulation of new products by the agrochemical industry is restricted to the use of approved substances, namely active ingredients (AIs) and other chemicals used as co-formulants, the approval of new substances requires time, testing and investment. Thus, the reformulation of available commercial products using authorized AIs represents a faster and viable option. In this context, two commercial pesticide products were used as models in this study to assess the feasibility of their reformulation towards safer alternatives concerning effects on non-target organisms (first stage) keeping efficacy against target species (second stage). One was an herbicide formulation combining the AIs bentazone and terbuthylazine, and the other a fungicide combining azoxystrobin and tebuconazole. Terrestrial and aquatic model species were used as non-target organisms in the first stage of testing for safety assessment, and a weed and two fungal species in the second stage as recommended targets of the model herbicide and fungicide products, respectively. Specifically, the AIs were tested at recommended and alternative doses/ratios with sensitive non-target organisms (oilseed rape *Brassica napus*; microalgae *Raphidocelis subcapitata*) to identify options with reduced impacts to the environment. Then both recommended and safer alternatives were tested against the weed (*Portulaca oleracea*) or fungi (*Pyrenophora teres* and *Rynchosporium secalis*) for efficacy assessment. Results showed that: (i) commercially recommended doses of the herbicide product represent a remarkable potential risk to soil ecosystems; (ii) application doses 10-fold lower than recommended are effective in the control of the target weed; (iii) a one-way formulation including only bentazone seems to represent an environmentally safer alternative to the two-way formulation in the control of *P. oleracea*; (iv) recommended doses of azoxystrobin and tebuconazole were not very effective in preventing the growth of the target fungi; and (v) there was not an apparent gain in the fungal growth control by combining these two fungicide AIs compared to single options. These results suggest that commercial pesticide formulations have room for optimization in order to improve their environmental safety while keeping efficacy against weeds/pests.

6.05.V-03 Ovarian Ultrastructure and Embryonic Development of the Fish *Poecilia reticulata* Exposed to Maghemite Nanoparticles (Y-Fe₂O₃) Associated With Glyphosate

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The synthesis of nanomaterials to act in environmental remediation and depollution of the aquatic environment indicated that the use of maghemite nanoparticles (IONP) is promising. But its ability to interact with glyphosate-based pesticides is still underexplored. Thus, to investigate the toxic effects on reproduction and embryogenesis in guppies, experiments were carried out with IONPs and those associated with GBH and GLY. Thus, 7 young and adult females of guppy (*Poecilia reticulata*) were exposed for 7.14 and 21 days in triplicate to the experimental groups with iron ions - IF (0.3mg / L) and IONPs associations (0.3 mg Fe / L) with GLY (0.65 mg / L) and GBH1 GBH2 (0.65-1.30 mg GLY / L, respectively), a control group (GC) was also considered, as well as post-exposure groups in which the same number of fish remained for an equal period in reconstituted water. The fish were euthanized, dissected, and the ovaries were fixed in 2.5% glutaraldehyde and post-fixed in 1% OsO₄, dehydrated in acetone concentrations, critically point dried (Leica EM CPD300), covered with a 20nm layer of gold in the sputtering (Leica EM SCD050) and analyzed under a scanning electron microscope (JEOL JSM IT300LV – CRTI / UFG). The ultrastructural analyzes considered the treatment/time relationship. Multiple embryos were observed at different stages, therefore, development was asynchronous and took place in waves of embryonic maturation. There was an indication of recovery in the groups exposed for the same time to reconstituted water. But the treatments promoted changes in the connective tissue structure that involves the ovary, both externally and internally, being more intense after 14 days for the IF and GLY groups. In IONPs+GBH, the alterations

were smaller, suggesting that they are larger elements and had difficulty in transposing the conjunctival barrier. Exposure to IONPs induced reproductive toxicity in female guppies, it was observed that in embryos at early and intermediate stages of development, the chorion had disorganized microfolds when compared to GC, and the outer layer of oocytes had alterations in the structure of the fibril complex of the inner layer. These analyzes demonstrated the importance of evaluating the development process in order to understand the biological characteristics of the species and indicate the capacity for preservation in vulnerable environments, which may be the target of remediation by maghemite.

6.05 Mixtures matter – from science to regulation and management (Poster)

6.05.P-We268 A Battery of EcoToxicological Tests to Assess Environmental Risk After Utilization of Mineral Fractions From Incineration Bottom Ash Treatment

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At EU level, neither End of Waste criteria, nor a harmonized methodology for establishing the requirements for the utilization of the mineral fraction of bottom ash in construction have been issued. To promote the utilization of BA, an evaluation of the potential risks that these materials may pose to the environment and human health is needed. The aim of the project was to use a battery of aquatic and terrestrial ecotoxicological tests to characterize the risk after the use of End of Waste material from incineration BA. Ten different samples provided by four different plants were analyzed. Each sample was divided in three aliquots and the column percolation test according to EN 14405 was carried out on each aliquot. The eluates obtained were employed in the aquatic ecotoxicological tests, performed according to OECD 201 Freshwater Alga and Cyanobacteria Growth Inhibition test, OECD 202 Daphnia sp. Acute Immobilization test, OECD 203 Fish Acute Toxicity test and OECD 211 Daphnia magna Reproduction test. The tests were performed at 5 different concentrations, starting from a 1:5 dilution of the original eluate. Terrestrial ecotoxicity tests were performed on the samples that were ground to obtain a particle size of 1 mm, to grant a better homogenization with the soil. Five different concentrations ranging from 100 to 1000 mg/kg d.w. have been tested. To cover different ecological level, the tests selected were OECD 208 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, OECD 216 Soil Microorganisms: Nitrogen Transformation Test, OECD 222 Earthworm Reproduction Test, OECD 207 Earthworm, Acute Toxicity Test, OECD 232 Collembolan Reproduction Test in Soil and OECD 226 Predatory mite reproduction test in soil. The EC₅₀ was derived for all the acute tests and the NOEC and EC₁₀ for all the chronic tests. To perform the aquatic risk assessment, the concentrations expected in the groundwater and in surface water were estimated using standardized fate and transport models that account for the attenuation during the leaching in the unsaturated zone and the dilution occurring in the groundwater and in surface water, considering three different utilization scenarios. These concentrations were compared with the endpoints derived in the aquatic toxicity tests and at least one safe use was defined for each sample. For the terrestrial risk assessment, a weight of evidence approach was used to evaluate the potential risk based on the obtained results.

6.05.P-We269 Analysis of Cumulative Risks of Unintentional Mixtures in Europe

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Unintentional exposure to chemical mixtures might be a problem, as mixtures are generally insufficiently acknowledged in chemical risk assessment. Therefore, the EU commission suggests a preventive (pro-active) approach in the form of a Mixture Assessment Factor (MAF, also referred to as Mixture Allocation Factor) to account for these risks. To stimulate the debate, this study (1) evaluated the need for addressing unintentional co-exposures to multiple chemicals in chemicals risk assessment by quantifying the fraction of water bodies that is insufficiently protected, and (2) explored the effect of fractional reductions of exposure concentrations on improving the percentage of water bodies that is sufficiently protected. We curated and aggregated EU surface water monitoring data from the NORMAN network (>9.5 million chemical measurements) to derive ambient chemical mixtures. For these mixtures, the total toxic pressure was calculated using several different approaches, including mixture Risk Characterization Ratio's (RCRs) and multi-substance Potentially Affected Fractions of species (msPAF) under several assumptions. In addition, we applied simplified scenarios to obtain insights on the effects of potential fractional exposure reduction on environmental quality. The results indicate (1) that water bodies are insufficiently protected against unintentional mixtures, (2) that a relative small fraction of chemicals (e.g. ~10%) dominate the ambient mixture toxic pressure (e.g. ~85%), and (3) that low fractional reductions in exposure concentration can result in a steep increase of the percentage of water bodies that are sufficiently protected. Although the monitoring data do not represent the full spectrum of real-life ambient mixture exposures, the results appear to be robust under several other assumptions. Accordingly, the results indicate the need for adapting policies to reach European Union goals for a toxic-free environment and underpin the utility and possible magnitude of a Mixture Allocation Factor.

6.05.P-We270 An Assessment of Chemical Risk of Unintentional Mixtures at the EU Continental Scale Based on Aquatic Chemical Monitoring Data

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Existing regulatory frameworks in the EU have proven to be effective in identifying and mitigating chemical risk. However, there is growing interest in understanding to what extent mixtures of chemicals from different regulatory domains may result in

additional risk. We analyzed the potential additional chemical risk from unintentional mixtures of chemicals from multiple domains in EU freshwaters by performing a screening level chemical mixtures risk assessment at the continental scale. Our analysis combined the largest publicly available chemical monitoring database in the EU: the Waterbase Waterquality ICM database, with state-of-the-art ecological effects metrics and chemical mixture assessment methods. The investigation included more than 330 chemicals across pesticides, pharmaceuticals, metals, and industrial chemicals. The evaluation included 22 EU countries, more than 4000 monitoring sites in more than 4000 waterbodies. Central elements of our approach were (1) the classification of chemical monitoring observations by the type of chemical risk, and (2) the determination of the number and identity of main chemical risk drivers. We found that most monitoring samples did not pose a chemical risk concern from single substances or mixtures (92.92%). The percentage of samples whose risk was dominated by complex mixtures was very low (less than 3% in all cases). We found that the three first chemical risk drivers per sample contributed on average a 90% of the total risk, and the first risk driver alone contributed on average a 50% of the total risk per sample. The combined contribution of all other chemicals present in the mixtures was on average less than 10% of the total risk per sample. We identified that a very specific set of chemicals (about 2% of all measured chemicals) drove most of the risk in the entire dataset. Our findings provide critical evidence on the prevalence, characteristics, and drivers of the chemical mixtures risk found in “unintentional mixtures” currently in EU freshwaters.

6.05.P-We271 Short-Term Chronic Toxicity Test for Assessing the Adverse Effects of Chemical Mixtures Using Zebrafish Embryos

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With increase of usage and production of household chemical products, there is rising public concerns about the hazards of chemical mixtures in wild lives, including human. Methodological approach for determining the potential effects of chemical mixtures should be assumed that the mixture profile of environmental chemicals and the dissolved levels of the individual constituents are preserved between the toxicity tests. In this study, mixture toxicity test with zebrafish embryos was conducted to investigate the adverse effects profile induced by exposure to different chemical mixture combinations, within short-term period. Toxicity values of chemical mixtures were estimated by the effective concentration (EC_x) focusing biological response index during during the embryonic developmental phase in zebrafish. The adverse effects profile from chemical mixtures was examined by the transcriptional alteration of target genes related to cellular damage and development with phenotypic analysis of embryonic development. The embryonic developmental toxicity of chemical mixtures was relied on the interactive effects between individual constituents due to mixture combinations. Consequently, our results provides toxicological information regarding the impacts of chemical mixtures, such as household chemical products, using the embryonic development of zebrafish. This methodological approach applied transcriptional alteration of target genes with phenotypic analysis can serve a response biomarker for assessing the environmental hazards of chemical mixtures.

6.05.P-We272 Ecotoxicological Drivers in Bio-Hybrid Fuel Development

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Increasing industrialization, standard of living and population are leading to an increase in global energy and fuel demand, particularly in the transportation sector. While changes in propulsion method challenge the relevance of internal combustion engines, liquid fuels will remain a relevant form of energy carriers in many regions and for many applications in the future. While the use of petroleum-derived diesel and gasoline fuels poses a significant risk for the environment, human health and global climate, the introduction of sustainable, less toxic fuels provides an opportunity to avoid or reduce harmful effects. This is especially important considering the large amount of fuels required by the transportation sector and the consequential contamination of the environment by fuel spills and leakage. Therefore, research on developing alternative and renewable fuels is increasing. Bio-hybrid fuels represent promising alternatives to fossil fuels. They are generally considered to be more environmentally friendly and more sustainable than fossil fuels and contribute less to global climate change, especially when produced with excess renewable energy. The new regulatory focus regarding mixtures based on the EU Chemicals Strategy for Sustainability is highly relevant for the future of Bio-Hybrid fuel blends and needs to be considered accordingly. In this study, toxicity assessment of bio-hybrid fuel candidates and blends thereof is integrated in the fuel development process of a novel ketone-ester-alcohol-alkane blend (KEAA blend) following the previously proposed framework for a “Green Toxicology” approach. The KEAA blend consists of six constituents (methyl isopropyl ketone, ethanol, methyl acetate, ethyl acetate, pentane and methanol) and has recently been identified as a possibly superior fuel for spark ignition engines. Toxic potential towards aquatic organisms of blend constituents and the blend itself is assessed *in silico* and *in vivo*. By modifying the blend to trigger lower toxic effects, collaborators in chemistry and engineering gain insight into environmental optimization potential. By providing this information on potentially hazardous constituents early in the development process, further development can be focused on less toxic blend variants. This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany’s Excellence Strategy – Cluster of Excellence 2186 „The Fuel Science Center” – ID: 390919832.

6.05.P-We273 Risk-Based Screening for Prioritisation of Organic Micropollutants in Swedish Freshwater

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Concerns related to the contamination of the environment by organic micropollutants (OMP) are raising due to their potential

bioaccumulative and toxic properties. The aim of this study was to evaluate the risk posed by OMPs to the aquatic ecosystem in Swedish freshwaters. The assessment was based on measured environmental concentrations (MEC) of OMPs in surface waters upstream and downstream of Swedish wastewater treatment plants (WWTP). A novel optimized risk quotient (RQf) was used to identify potential high-risk substances in the aquatic environment. Among the 126 substances investigated, this study showed that 10 compounds are likely to pose a risk to the aquatic ecosystem (i.e. $RQf > 1$), and two compounds are posing a moderate risk to the aquatic ecosystem in Swedish surface waters (i.e. $0.1 < RQf < 1$). The data presented here could be used for assessing the effects of OMP mixtures in aquatic ecosystems, and ultimately by local and international authorities to prioritise OMPs and contaminated hotspots, in order to decrease the risk posed by these compounds. This study highlights the importance of the selection and the reliability assessment of ecotoxicological data for the calculation of predicted no-effects concentrations (PNECs), which are the basis to determine the risk quotients (RQs). It also critically evaluates the efficacy of this method globally used in chemical regulations to protect ecosystems.

6.05.P-We274 Suggestion for Mixture Assessment of Pesticide Concentrations Monitored in European Waters

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Different pesticides are regularly monitored by different member states for their unintended occurrence in surface and groundwaters of the European Union. The selection of pesticides for monitoring is based on different regulations, expert knowledge and political rationales. Monitoring data are subsequently submitted and collected in the publicly available WISE database (<https://www.eea.europa.eu/data-and-maps/data/wise-wfd-4>). Based on this database summaries are published such as for example yearly concentrations of the nationally selected pesticides for designated monitoring stations in different member states. One goal behind the substantial efforts of monitoring pesticides is to identify and manage those substances which are drivers of toxicity and thus contribute to the bad quality of European surface- and groundwaters. Research in ecotoxicology over the last decades demonstrated that organisms are often exposed and affected by contaminant mixtures rather than by single substances. Even if the different contaminants occur in low concentrations in the environment they may contribute to a joint overall effect ("something from nothing")- which can be predicted using the mixture concept of Concentration Addition (CA). Experimental evidence supporting the use of this mixture prediction approach and its use for chemical risk assessment has been reviewed and discussed by various institutions and authors, e.g. SCHER, SCCS, SCENIHR, Opinion on the Toxicity and Assessment of Chemical Mixtures, 2012 or Kortenkamp et al. (State of the Art Report, 2009). In light of this knowledge on mixture toxicity the current means of assessing monitoring data and deriving subsequent management for individual substances only may lead to an underestimation of existing risks. One goal of the work presented here was to suggest a way of how to make use of the multitude of pesticide concentration data in the WISE database for a mixture toxicity assessment strategy. Based on a toxic unit summation approach different ways of prioritizing are suggested. Ideally, this would be a starting point for enabling member state authorities and other stakeholders to prioritize and target the pesticides and pesticide groups for regulation or management (e.g. phasing out, reduction or replacement).

6.05.P-We275 Rural Emission to Kaldvellfjorden in Norway and Characterization of Cumulative Risk of Metals

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Kaldvellfjorden is located in the Lillesand area situated near the border of the Skagerrak Rift south in Norway and the fjord are impacted by acid rock drainage (ARD) due to construction work in the nearby sulfide-bearing bedrocks. Exposure of rock surfaces to air and precipitation have occurred over a long time, however the oxidation of the rock increased especially after the E18 road construction in 2006 and 2008, when also three large landfills of waste rock material were constructed. Especially landfill close to Kaldvellfjorden has contributed to high ARD and one treatment plant has been constructed to reduce the metal concentration in the ARD. Trace elements monitored in the fjord was subjected to a component-based Cumulative Risk Assessment (CRA) using the NIVA Risk Assessment database (NIVA RADb, www.niva.no/radb) to predict site-specific impacts. The resulting CRA demonstrated that fish had the highest risk (i.e. risk quotient) for acute effects in Kaldvellfjord, followed by molluscs and algae. For fish several elements were estimated to contribute significantly to the acute risk and in the following order $Fe > Zn > Cu > Cd$. Analyses of gills of caged Atlantic Salmon smolt exposed to the fjordwater showed significant accumulation of several of the elements identified to cause risk, but also accumulation of other elements not identified to cause risk due to lack of toxicity data for marine organisms. The observed data suggest that several of the trace metals including rare earth elements contribute to risk in Kaldvellfjord, and studies combining computational and experimental efforts presented herein may provide complementary information to impact assessments of complex exposure scenarios. Acknowledgement: This project was supported by the Research Council of Norway through project 268294: Cumulative hazard and risk assessment of complex mixtures and multiple stressors (MixRisk), Centre of Excellence funding scheme, project number 223268/F50 and NIVAs Computational Toxicology Program (www.niva.no/nctp).

6.05.P-We276 Addressing Mixture Related Policy and Research Priorities With Data From IPCHEM, the EU Information Platform for Chemical Monitoring

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The Information Platform for Chemical Monitoring (IPCHEM) is the EU reference access point for finding and retrieving chemical monitoring data. IPCHEM supports a coordinated approach for collecting, storing, sharing and assessing data on the occurrence of chemicals and chemical mixtures in humans and the environment. The data is structured in four thematic modules: environmental monitoring, human biomonitoring, food and feed, products and indoor air. The database currently incorporates over 450 million concentration measurements from over 3000 substances and 170 data collections. Data streams coming from periodic regulatory monitoring and research consortia is continuously integrated following defined quality and harmonisation procedures. By providing monitoring data from data collections covering multiple exposure sources IPCHEM supports the assessment of risks from combined exposure to multiple chemicals. Examples of mixture related policy questions that can be addressed with help of IPCHEM include: - the assessment of risks from combined dietary and non-dietary exposure to pesticides mixtures for human health; - the derivation of chemical mixture profiles and associated risks in the European population using human biomonitoring data representing internal exposure in the general adult population or in specific (sub)populations (e.g. children, pregnant woman); - the derivation of chemical mixture profiles and associated risks in the environmental media including surface water, drinking water, sediments, soil, air, etc.; - the development of exposure risk indicators for use in policy evaluation under specific regulatory instruments (sustainable use of pesticide directive) and the broader Chemical Strategy for sustainability; - Identification and characterisation of sources, routes of exposures, body burdens and associated health effects from real-life scenarios of indoor exposure to chemicals; These examples illustrate the importance of data harmonisation and interoperability as key features necessary to address chemical mixtures problems across regulatory domains. In fact, monitoring data becomes most useful when used in combination with additional exposures (e.g. biologicals) and other health and ecological data. For example, monitoring data combined with human health data (e.g. disease registries) or ecological monitoring (e.g. ecological stressors, biodiversity indicators) can feed into (eco)epidemiological studies linking exposure to effects.

6.05.P-We277 MEEDs: The Multiyear Metals Environmental Exposure Data Collection Program, Anticipating the Challenges of the EU Zero Pollution Ambition Policy and the Chemicals Strategy for Sustainability

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As part of the EU Green Deal, the European Union Zero Pollution Ambition aims at reducing exposures of chemicals to levels that are no longer expected to be harmful to health and the Environment. The Chemicals Strategy for Sustainability (CSS) is a pillar of this ambition. It will be implemented through revisions of key chemicals legislations like REACH and CLP bringing in new challenges like the Mixture Assessment Factor (MAF) to demonstrate safe use and lack of impact on ecosystems for the cocktail of chemicals exposures in environmental compartments. The volumes of metals in use are expected to increase in view of the role metals play in reaching the climate and circularity objectives of the Green Deal. Hence, it is crucial to demonstrate that exposure of metals and their mixtures in the receiving environment will meet the challenge of this ambition and the MAF, now and in the future. Therefore, the EU metals sector designed a comprehensive “environmental exposure gathering programme” (MEED), complemented by scientific concepts development to comply with the Zero Pollution Ambition and biodiversity objectives. The programme’s timeline (2022-2024) has been defined to feed the deliverables in due time into regulatory debates (e.g., REACH Revisions, MAF impact assessments and debates, Zero Pollution Action Plan activities, Water Framework Directive etc.). MEED consists of 6 interlinked projects covering a comprehensive update of the Regional and Sewage Treatment Plants (STP) exposure assessments to identify EU relevant time trends and perform source allocation. A specific module of MEED focuses on the ‘MAF’, by assessing metal mixture interactions for priority contributing substances and increasing understanding of metals-organics mixture interactions. Both the regional exposure and the MAF modules feed into an ecorelevance project to check the impact on biota under realistic EU exposure conditions. MEED will deliver a long series of data that can be used in REACH registration updates and other regulatory programmes.

6.05.P-We278 Metal Mixture Risks in European Soils: Support for a Tiered Metal Mixture Risk Assessment Framework

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Metal contamination in soils occurs generally as mixtures of metals. However, metal mixture risk assessment is complicated due to the influence of bioavailability on metal toxicity, natural background concentrations of metals, and for some metals also essentiality. The most basic mixture reference model available for assessing risks at the ecosystem level (CA_{SSD}: concentration addition applied at PNEC or the 5% hazardous concentration of the species sensitivity distribution) typically results in unrealistically high metal mixture risk predictions in soils. Several metals, such as Cu, Co, Ni, Zn, Mo, Pb, and Cd, are data-rich substances, which allows to consider the entire species sensitivity distribution in mixture risk assessment evaluations. More refined and theoretically more consistent models that apply the mixture reference models concentration addition or independent action at the dose-response curve (DRC) of each species individually prior to estimating the fraction of affected species in an ecosystem (i.e. CA_{DRC} and IA_{DRC}) exist. Recently, a tiered freshwater metal mixture assessment framework integrating these more refined models in higher assessment tiers was developed based on evidence from aquatic toxicity tests. Limited evidence from ecotoxicity tests with soil organisms supports the extrapolation of this tiered framework developed for freshwaters to soil ecosystems. Previous research suggested that the concentration addition model overestimated observed metal mixture toxicity at low effect concentrations (i.e. 10% effect concentration) with on average 3.6-fold across 10 different mixture experiments with *Hordeum vulgare*. To build support for the underlying assumptions of the tiered risk assessment framework for soil ecosystems, the conservativeness and general performance of the mixture reference models IA and CA in predicting chronic mixture effects to soil organisms was evaluated in a systematic manner based on published studies. Relevant research questions that were addressed are I) Which reference model is most accurate for predicting chronic metal mixture toxicity to soil organisms; II) How frequently

do statistically significant deviations relative to IA and CA occur; III) How accurate is the standard CA approach for predicting mixture effects at low effect concentrations. In addition, in-silico metal mixture risk predictions will be used to evaluate the order of conservatism of the existing metal mixture risk prediction models (CA_{SSD}, IA_{SSD}, CA_{DRC} and IA_{DRC}) at the community level for soils. The results of the present study will further support the use of a tiered risk assessment framework for metal mixture in (European) soils.

6.05.P-We279 Metal Mixture Risks in European Soils: Application of a Tiered Metal Mixture Risk Assessment Framework for Arable Soils in Europe

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Metal contamination in soils occurs generally as mixtures of metals. However, metal mixture risk assessment is complicated due to the influence of bioavailability on metal toxicity, natural background concentrations of metals, and for some metals also essentiality. Given these metal specific characteristics, the most basic mixture reference model available for assessing risks at the ecosystem level (concentration addition applied at the applied at the PNEC or 5% hazardous concentration of the species sensitivity distribution) typically results in unrealistically high risk predictions in soils. Previous research suggested that the concentration addition model overestimated observed metal mixture toxicity at low effect concentrations (i.e. 10% effect concentration) with on average 3.6-fold across 10 different mixture experiments with *Hordeum vulgare*. Based on this observation and similar observations for chronic metal mixture toxicity to aquatic organisms, a tiered metal mixture framework has been developed combining the mixture reference models concentration addition and independent action with either the SSD and PNEC or the single-species dose response curves. The current study applies this metal mixture risk assessment framework on the GEMAS database, a soil database with measured metal concentrations representative for European exposure in arable soils (2108 individual samples under normal agricultural production) to assess the potential risks associated with metal exposure (Cu, Ci, Ni, Zn, Mo, Pb and Cd). All SSDs were fitted with a log-normal distribution and bioavailability corrections were performed according to models reported in the chemical safety reports. Of the 2108 sampling points, 15% were predicted to be at risk by exceedance of the environmental threshold of at least one metal (Tier 0). On the other hand, 36% of the samples are predicted to show no potential risks based on the most conservative model (CA_{SSD}), while 49% of the samples would be considered to be at potential risk due to mixture toxicity when only CA_{SSD} would be considered as mixture risk assessment method (Tier 1). For these samples, more accurate approaches applying either concentration addition or independent action at the species level are integrated in higher tier levels to further refine the metal mixture risk assessment. The current study shows how bioavailability normalized species sensitivity distributions-approaches for metals can be combined with the mixture reference models concentration addition and independent action to refine conservative metal mixture risk predictions in a tiered risk assessment framework using a representative European agricultural soil database.

6.05.P-We280 What Are the Main Drivers That Contribute to Herbicidal Mixture Toxicity in a Landscape That Is Highly Influenced by Agricultural Practices?

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In the natural environment chemicals rarely occur as single entities but in undetermined mixtures. Despite this, the environmental risk assessment for plant protection products (PPPs) within the EU focusses on single substance registrations, except for products containing more than one active ingredient. Freshwater bodies in urban and agricultural areas may harbour an infinite number of combinations of anthropogenic chemicals. The assessment and regulation of these mixtures has raised concerns and prompted discussions by researchers, the public and regulators for at least the last three decades. These have included the implementation of a mixture assessment factor (MAF), but also more differentiated approaches e.g., addressing potential risks by managing critical compounds in spatio-temporal hotspots and their common mode of action (MOA). It has been demonstrated that only a limited number of chemicals drive the mixture toxicity, i.e., mixtures are not equitoxic. For photosynthetic organisms, mixture toxicity is primarily related to herbicides. A monitoring program carried out for 3.5 years in a highly agriculturally influenced catchment included sub-daily sampling of 12 herbicides and 1 metabolite. Here, we assess the relevance of this herbicide mixture in surface waters and propose how the data could be used for future regulatory decision-making processes. We identified how often the mixture exceeded the regulatory accepted concentration (RAC), assuming additive toxicity, which herbicide(s) contributed most to the exceedance events and determined if any factors could be identified contributing to these events (e.g., flow, rainfall, seasonality). Additionally, a stewardship program added to the influencing factors by implementing differing farming techniques and avoidance of missuses, spillages, and other point sources during study. The data were analysed separately for algae and macrophytes and although there are a low number of common herbicides driving the potential toxicity in both groups, there were also distinct differences where one group was more sensitive to a compound than the other (i.e. lower RAC). We found a clear seasonal influence driven by the growing season and rainfall and a stark reduction of exceedance events for both algae and macrophytes during the stewardship program. Overall, the analysis illustrates how monitoring projects can enhance the knowledge of the influencing factors that will enable improved decision making in the future.

6.05.P-We281 Masking Persistence - How Solvents Give a False Sense of Security With Respect to Biodegradation

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The aquatic environment is subject to multiple chemical inputs. Many of these chemicals are readily biodegradable, but some are

not. Chemicals such as emulsifiers, flow improvers, paints, tracers, other mixed substances and unknown or variable composition, complex reaction products or of biological materials (UVCBs) have highly biodegradable components such as solvents. Some solvents in mixtures are volatile organics, others are organic acids that increase solubility through pH changes. These not only enhance solubility and handling but also enhance biodegradation potential to exceed regulatory thresholds and thus avoid substitution warnings, even where part of the substance is persistent. Under most regulations the definition of a substance includes “a chemical element and its compounds, in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.” For substances such as polymer emulsions this is ambiguous and the presence of solvent can allow it to be falsely identified as readily biodegradable, or at worst moderately persistent due to the reactions of the solvent. Once a substance enters the aquatic environment it is diluted and dispersed and smaller mobile molecules, such as those of solvents are stripped out of emulsions or mixtures, through microfluidic interaction at the molecular level, or through volatilisation/evaporation. The smaller molecules are then more readily available for biodegradation, leaving the polymers, or more recalcitrant solutes behind to potentially build up and pollute the environment. Solvent masking of persistence is being addressed by OSPAR’s offshore industry committee after a UK proposal was ratified in 2019. There are several ways of determining biodegradability of substances, either by evaporating volatile solvents from mixtures making them harder to handle and reducing the distribution in a biodegradation test, or by solvent subtraction methods. Either of these approaches will increase the number of substances that are classed as ‘substitutable’ but they represent a more realistic way of classifying the chemicals environmental hazard. Here we use a solvent subtraction method to demonstrate the effect of masking persistence due and consider the implications of such masking.

6.06 Novel Life Cycle and Sustainability Assessment approaches supporting biodiversity in land and water management

6.06.T-01 A Regionalized Species-Discharge Relationship for Assessing the Impact of Water Consumption on Freshwater Biodiversity Globally

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Freshwater biodiversity is undergoing a mass extinction driven by multiple environmental pressures, including altered river discharges and flow regulation. Existing end-point LCIA methods focusing on freshwater in-stream species richness have modelled the effect of discharge reduction on fish species richness using species-discharge relationships (SDRs). However, their restricted geographical scope and the small amount of species data underlying the models hinder characterization factor (CF) deployment at the global scale, which are needed to assess and compare the environmental impacts of global value chains. A working group within the Global Life Cycle Impact Assessment Method (2020-2023) project of the life cycle initiative hosted by UN Environment works on recommendations for impact assessment of water consumption on biodiversity. The group has developed a new SDR model, i.e., an effect model, with an extended geographical scope. This extended SDR model builds on updated datasets covering 11,500 fish species, simulated river discharges, and other relevant predictors. This model is then used to derive regionalized marginal and average CFs for water consumption impacts on freshwater biodiversity, along with associated uncertainty range estimates. The new SDR model includes river discharge, elevation and climate zones, thus reflecting two large-scale species richness gradients. It was selected among 5 model candidates supported by literature and a 10-folds cross-validation procedure. When testing the SDR robustness on independent observed river discharge data, the goodness of fit was higher (Kling Gupta coefficient $KGE=0.6$) than from previous global SDR employed in CF calculations ($KGE=0.13$). The effect factors were integrated with available fate factors and global extinction probabilities to obtain CFs. Marginal and average characterization factors are provided for 1,444 and 1,999 river basins, respectively, where water consumption has reduced the discharge flow and the SDR model is valid. The updated SDR-based CFs provide a more accurate estimate of the potential damage of water consumption on freshwater in-stream biodiversity than previous approaches. They cover more river basins and fish species, as well as specify marginal and average impacts.

6.06.T-02 GLAM Recommendations for Translating Regional Species Loss to Global Loss Within Life Cycle Impact Assessments

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Life Cycle Impact Assessment (LCIA) approaches have made substantial advances in modeling and incorporating spatially explicit environmental impacts on the rate of species loss within a particular area (i.e. “ecosystem quality” area of protection). Despite these advances, there are still gaps in the species coverage and opportunities to more accurately scale damage estimates. A fundamental issue that needs to be addressed is to improve the consistency of damage estimates between impact category indicators. Most state-of-the-art models quantify species loss at the local or regional level. However, for comparisons of global value chains, it is likely more appropriate to incorporate global extinction probabilities rather than regional losses only. We therefore need a consistent scaling approach that translates regional species losses to global species losses (i.e. global extinction).

A working group within the Global Life Cycle Impact Assessment Method (GLAM) project of the life cycle initiative hosted by UN Environment is developing recommendations for such an approach. The working group develops recommendations of how to scale regional to global species losses, within an LCIA framework, based on the Global Extinction Probability (GEP) approach defined in Kuipers et al., 2019). This approach used information on species range areas and the current threat level of the species compiled from IUCN Red List to calculate a scaling factor for translating regional species losses to global species losses. The working group built upon this approach by nearly doubling the number of species covered. The Global Extinction Probability factors can be applied consistently across impact category indicators that report impacts as (regional) “potentially disappeared fractions of species“ (PDF) within the AoP ecosystem quality for scaling them to global extinctions. The GEP factors can be calculated at any chosen spatial unit that is relevant for the impact category in question and with the relevant species groups, and thus provides flexibility to address specific issues. This approach contributes to harmonizing LCIA results. Reference: KUIPERS, K. J. J., HELLWEG, S. & VERONES, F. 2019. Potential Consequences of Regional Species Loss for Global Species Richness: A Quantitative Approach for Estimating Global Extinction Probabilities. *Environmental Science & Technology*, 53, 4728-4738.

6.06.T-03 Sustainable Fisheries: Towards Operationalization of Decision Making Accounting for Biodiversity

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Rationale: Overexploitation of biotic resources constitutes a major threat on marine biodiversity while demand for seafood will rise in the next decades. Application of Life Cycle Assessment to marine ecosystems needs further research to allow a quantitative characterisation of the impact of sea-based products on biodiversity. Moreover, there is a rising demand for product eco-design, as illustrated in Business @ Biodiversity studies; presently, corporates and policy makers do not have proper tools to decide for sustainable practices regarding seafood production. **Introduction - methods:** This study aims to apply existing assessment methods of the impact of overexploitation on biotic resources (Langlois et al. 2014 and Emanuelsson et al. 2014) to 125 marine stocks fished in the 14 marine areas drawn by the FAO. We present how the results can be reproduced, including what kind of database can provide for the data needed. We discuss how results can be interpreted as a proxy for biodiversity assessment and the ecological limits of the methods. Finally, we propose operational guidelines for sustainable production and efficient conservation policies. **Results:** We show that unsustainable fishing is responsible for a loss of up to 30 times the potential yield of major fish stocks such as Atlantic cod, red snapper and bluefin tuna. We identify depleted fish stocks for which biomass is up to 15 times lower than it should be (yellownose skate; South America) and stocks facing fishing mortality up to 1.9×10^7 times higher than the maximum required to allow sustainable recovery (Pacific Ocean perch, US West Coast). Regarding intrinsic biodiversity, our study shows that we are not able to understand the consequences of overfishing through a cause-effects chain due to lack of science knowledge. However we display how to limit the impacts on biodiversity by using complementary indicators at species and ecosystem level. **Recommendations:** While methods seemed to compete against each other in the impact assessment of marine stock overexploitation on biodiversity, this study shows their complementarity. Hence, in the aim of making seafood more sustainable, a combination of the indicators they provide should be used. The complexity of marine ecosystems and the remaining limits of methods are discussed, showing the compelling need for further data collection and analysis, and opening ways for targeted research.

6.06.T-04 Assessing the Environmental Profile of Sustainable Agricultural Production Systems Towards Biodiversity and Climate

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Agricultural activities have generally major implications on land use and emission of greenhouse gases. Both, land use and climate change have been identified among others as key direct drivers of biodiversity loss. Within the agricultural production context, besides chemical products numerous other tools and methods are used by farmers with the aim to enable a successful harvest and sustainable system. Consequently, the combination of those tools and methods create the major interface to the surrounding nature. The overall decision-making, choice of tools for a specific cropping situation and field context then decides about the more nuanced sustainability profile and the effects on habitats and the environment. The corresponding sustainability and environmental implications of field crop production have been investigated on two farms in Germany. The simultaneous use of selected sustainability metrics in the field trials allow differentiation and therefore can optimize decision-making for various cropping scenarios, increase transparency about environmental synergies but also about trade-offs, not only for the farmer but also within a regulatory context. The key metrics used in this work include crop yields per ha to reflect the land use footprint, the crop quality, an economic assessment of the production scenario, greenhouse gas emissions, biodiversity of carabids as proxy for natural pest control and biodiversity, soil health related to nutrient balance, organic carbon, earthworm populations and biodiversity of plants in habitats adjacent to the fields. The results of the production of winter wheat from the first year of the crop rotation are presented. The sustainability profile, climate and environmental implications of different production scenarios with their corresponding choices of tools and methods have been simultaneously assessed, thus enabling a holistic perspective that takes into account the farmers local agronomic challenges, decision-making and opportunities for improvement. Therefore, the presented approach can inform strategic decisions and outcome-oriented regulations, that help to improve the sustainability profile of agriculture and also drive solutions from an applied nature conservation perspective.

6.06.T-05 SETAC Workshops: "Optimising Agricultural Food Production & Biodiversity in European Landscapes"

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A series of four workshops were organised under the auspices of SETAC Europe dealing with the topic “Optimising crop production and biodiversity in European landscapes”. Stakeholders from industry, academia, government authorities, farmers and NGOs discussed potential pathways for more sustainable agricultural production. In this talk the approach, concepts and results of these workshops are presented. The overarching question, still not fully answered, is how to maintain efficient food production while improving the influence (+ vs. -) of agriculture on biodiversity. The objective of this series of workshops was to contribute to the ongoing debate by helping to inform decisions on multiple levels for the future management of agricultural landscapes, and more particularly, how to achieve the double objective of biodiversity protection and food production. A set of general principles was identified and agreed upon which should be considered when evaluating the various approaches and measures aimed to foster biodiversity in agricultural landscapes while securing agricultural food production. Four scenarios (cropping systems in landscapes) were identified as examples, for which suitable combinations of approaches were developed. A number of approaches are already available and it was possible to identify “low hanging fruits” and hurdles for implementation. Communication and trust building was key in the building of the scenarios with a common objective in mind. Targeted solutions that are fit-for-purpose are needed, through a suite of approaches - in field, on farm, off farm - that consider socio-economic context. i.e. whole system approach. The full proceedings of the workshop will be published in 2022.

6.06 Novel Life Cycle and Sustainability Assessment approaches supporting biodiversity in land and water management (Virtual Only)

6.06.V-01 Biodiversity Damage Assessment Integrating Carbon, WATER and Land Footprint

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1. Introduction The 2021 and 2022 Convention on Biological Diversity conference will be held. As a result, new targets for biodiversity will be set and quantitative assessments will be required. In particular, TNFD will be launched in 2021, and companies will disclose their natural capital assessment in 2022. Much of the natural capital to be assessed comes from food, construction, and energy, so it is extremely important to ensure proper procurement of these raw materials in countries where they are shared. 2. Materials and methods Multi-regional input-output tables are expected to be extremely useful in implementing the biodiversity footprint. Although there are several multinational input-output analyses, eora developed by the University of Sydney will be used. In order to consider climate change, land use and water, we will obtain CO₂ emissions, land use area and water consumption for each sector in Japan. Impact assessment will be conducted using LIME3, which allows analysis that reflects environmental conditions in countries around the world. Using both, damage analysis including climate change, land use and water consumption will be conducted. This will enable us to conduct a biodiversity footprint through the final products and services provided in Japan. The results from this study are also intended for use by Japanese companies. In cooperation with Japanese companies, we will try to conduct a footprint analysis of their products and implement a biodiversity version of SCOPE3.

6.06.V-02 Comparing LCIA Methods to Support the Farm to Fork Strategy in Assessing the Biodiversity Impacts of EU Food Consumption

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Food production and consumption is the major contributor to global biodiversity loss. The EU Farm to Fork Strategy is targeting the enhancement of sustainable practices to halt current biodiversity loss rates in alignment with the EU Biodiversity Strategy for 2030. These strategies highlight the role of supply chains in transboundary effects and the need to have a consumption perspective when assessing and monitoring the evolution along time. The Consumption Footprint - Food indicator, developed by the European Commission-Joint Research Centre, is a full bottom-up Life Cycle Assessment (LCA)-based indicator that can be employed to identify current biodiversity hotspots, as well as to monitor impacts of the EU food system along time. However, the assessment of biodiversity impacts is still under discussion in the LCA community. The goal of this paper is to assess the biodiversity hotspots of the EU Food system by identifying convergent messages among available LCIA methods to assess biodiversity loss. Eight different LCIA methods and derived characterization factors from other approaches for quantifying biodiversity impacts were compared: Recipe 2016, Impact World+, LC-Impact, Eco-scarcity, Stepwise, a land use intensity-specific model, and two approaches derived from GLOBIO. The Consumption Footprint – Food includes 46 different representative products (i.e., covering 85% of EU food consumption). The year 2015 was analysed in this study. Preliminary results highlighted the role of animal-based products in the biodiversity impacts of the EU food system. Land use and climate change were the major drivers to the overall biodiversity impacts, apart from environmental pollution which had the major role in LC (mainly associated due to the conversion to a common unit, from volume to area). The comparison of operational LCIA methods and a detailed hotspot analysis allows for supporting EU policy-making by identifying hotspots, as well as providing metrics to monitor progress against policy targets. Due to the lack of harmonization, this study compared available methods to provide convergent and more robust messages. Agreement is still required in the LCIA community regarding biodiversity assessment and this study can support further development by analysing the underpinning causes in divergent messages.

6.06.V-03 Life Cycle Assessment Characterization Factors for Land Use Impacts on Biodiversity in Organic and Conventional Farmland in the European Mediterranean Biome

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Biodiversity loss is on the rise mainly due to one principal driver, agriculture. Agricultural practices such as nutrient input, pesticide use, field operations and field cover, are closely linked with biodiversity, and organic agriculture (ORG) is often seen as one possible solution. However, there remains much difficulty in assessing biodiversity due to its complexity and high specificity on a local scale. The life cycle assessment (LCA) methodology is often used to address this complexity using characterization factors (CF) that predict the potential disappeared fraction (PDF) of organisms in specific land use types and intensities. However, previous LCA studies address intensity with a quite general approach, with broad land use types (e.g. cropland) and intensity levels (e.g. light, intense). Specific CF for ORG and conventional (CONV) arable and pastoral farming exist but are limited to the temperate and mixed forest biomes in Europe, excluding the Mediterranean regions. Moreover, no biodiversity CF are available for permanent ORG and CONV crops. To fill these gaps, new midpoint occupation CF were calculated expressing PDF of vascular plant species per m² in ORG and CONV arable and permanent crops in the European Mediterranean biome. These CF were based on literature studies for plant species richness within ORG and CONV farmland across four European countries. The CF were able to distinguish between different land use types (permanent, arable, hedges, grass strips) and management practices (ORG & CONV) in arable crop systems. However, based on the available studies, CF could not be differentiated in permanent crops, since they were highly dependent on the intensity of management practices, despite if it was ORG or CONV. The CF for ORG and CONV Spanish olives and Italian vineyards were not significantly different due to the extensive management practiced in the CONV farms. Whereas CF for Spanish vineyard and Greek olive production could differentiate between ORG and CONV agriculture due to intensive practices in the CONV farms sampled. Finally, the performance of the new CF was tested using the mean yield data from the same literature studies used to calculate the CF as a simple example to show how the PDF results could be interpreted. CF derived from real field measurements of species richness ensures higher certainty of the results, and given that more data on biodiversity is becoming available, further CF can be calculated using this method.

6.06.V-04 Magnitude of Differences in Characterization Factors for Terrestrial Biodiversity in LCIA: An Overview of Regionalized Research

pınar kavak gulbeyaz and Hatice Şengül, Hacettepe University, Turkey

Regionalized Life Cycle Impact Assessment (LCIA) is a field that needs further research to improve LCIA methodology and practice in order to reduce uncertainty of LCA results. Regionalized research is specifically important for assessing biological diversity due to Land Use and Land Cover Change (LULCC). To date, various methods have been proposed for presentation of characterization factors mainly at the country and ecoregion scales. However, at these scales of assessment, local differences are neglected due to aggregation or averaging of damage factor values. In order to increase the accuracy of the impact assessment, quantification of impacts at smaller spatial scales is needed to determine the differences between local and larger scales. In this review study, we collected current characterization factors presented in peer-reviewed publications, aiming to check the magnitude of differences between evaluations at different spatial scales with the hypothesis that identification of case specific and site specific impacts is important and likely to result in different results from assessment at larger scales. This hypothesis is proven in the literature, however the degree of the difference is not fully covered and evaluated. In order to fill this gap, we performed a literature search using Google scholar and Web of Science databases with relevant keywords and Boolean operators to identify published characterization factors. Comparison among available characterization factors and examination of how local impacts relate with the country or ecoregion scale impacts were evaluated. Emerging themes were identified and compared with themes of core research that links biodiversity and LULCC. Conclusions of the review may provide insights to practitioners for decision-making and evaluation of possible variations.

6.06 Novel Life Cycle and Sustainability Assessment approaches supporting biodiversity in land and water management (Poster)

6.06.P-Tu301 Between the Lines: Quantifying the Life Cycle Impacts of Power Lines on Norwegian Biodiversity

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Recent life cycle impact assessment models have started to consider the impacts of renewable energy production on biodiversity, for instance hydropower and onshore wind-power plants. Although the transition to renewable energy plays an important role in the development of electric grid, the effects of electricity distribution on ecosystems and biodiversity are not addressed to date. Power lines may be considered only as affiliated infrastructure to energy generation, yet their impacts on wildlife are widely known: their construction and operation induce disturbance to species, collision and electrocution risk for birds, landscape fragmentation and habitat loss. We developed four impact pathways to assess the impacts of power lines on birds: collision, electrocution, disturbance and habitat degradation. For collision impacts, we calculated a collision probability risk based on birds' species-specific morphological characteristics (i.e. wing loading and wing aspect). Habitat suitability maps and grid data were used as a basis to quantify species richness loss by the potentially disappeared fraction of species metric (PDF). A final conversion of local PDFs to a global scale with global extinction probabilities indicates species vulnerability. The results of the study show how impacts of power lines vary spatially, identifies important areas for biodiversity and indicate which bird species are most susceptible to collision in Norway. The application of the developed methodology to other geographical areas is possible based on data availability. It can contribute not only to the promotion of sustainability of energy systems within Norway, but also worldwide. An evaluation of the overall biodiversity impacts of both energy production and transmission within the electricity supply-demand network will contribute to a better understanding of how renewable energy can harm biodiversity nowadays and in

the future. Thereby, providing an opportunity to assess how renewable energy can be promoted, while its impacts on biodiversity are minimised.

6.06.P-Tu302 The MarINvaders Tool : A Basis for Marine Biodiversity Impact Assessment and First Step on Closing an Important Gap in Life Cycle Impact Assessment

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Life Cycle Assessment (LCA) is a recognized tool for holistic assessments of environmental impacts, but to this date impacts from marine invasive species have not yet been covered by the LCA framework, even though invasive species cause major impacts on the marine environment. When not included in the LCA framework, decisions and policies based on LCA are likely to overlook these impacts on marine ecosystems. As a first step to overcome this gap, we have collected spatial data on threatened, native and alien marine species in a database and visualized the data in a web toolkit we call MarINvaders. The Ocean Biogeographic Information System (OBIS) is the main building block of our database. Species data from OBIS is used to identify which species are found within each ecoregion. The marine ecoregions defined by Spalding et al. provide the geographic unit of species location. The World Register of Marine Species (WoRMS), the Global Invasive Species Database (GISD), and the Nature Conservancy database on marine invasive species (NatCon) were used to identify the names and locations of marine alien species. The IUCN red list was used to identify which species are threatened by invasives. The MarINvaders database covers 96% of the 232 marine ecoregions and includes 108,787 species. Of these, 1,655 are impacted by invasive species and 909 are alien. We found that the fraction of species which are alien are dominated by fish (*Actinopterygii*, 137), *crustaceans* (140), and red algae (*Florideophyceae*, 71). We also found that more alien species are found in developed regions. The MarINvaders tool can be used for statistical analysis, like where the highest fraction of alien species is found compared to the total number of species or how many native species are affected per alien species. The database is an up-to-date collection of the spatial range of alien and native marine species and marine species threatened by invasives and can be used to identify management strategies of species that are already known to be invasive. Impact assessment modelling within the LCA framework needs global comparable data on the alien and native range, introduction pathways, and impacts of invasives - the MarINvaders tool so far fulfils the first two prerequisites and aids in finding and modelling the two latter. This makes it a basis for future modelling of regionalized LCIA characterisation factors for impacts of marine invasive species on marine ecosystems.

6.06.P-Tu303 Optimising Crop Production and Biodiversity in European Landscapes - a Task for All Stakeholders Involved. Interest Group of Regulators Started

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Under the auspices of SETAC Europe, a series of four workshops were organised over the last years (2020-2021) dealing with the topic "Optimising crop production and biodiversity in European landscapes". Stakeholders from industry, academia and authorities as well as farmers have had fruitful discussions and summarised potential pathways for a more sustained agriculture. Participating regulators decided to start an informal follow up activity to discuss possible fields of actions for improvement from a regulatory perspective. One outcome was that networking between the regulators offers opportunities to learn from one another. Therefore, the authors [1] of this paper aim to initiate a broader exchange and discussion among regulators from European Member states. On this poster, possible fields of actions for regulatory initiatives are identified. Even though the members of the group work in the field of crop protection and plant protection products (PPP) the agenda of this group is to cover a holistic perspective towards production and biodiversity and on how the regulation of PPP is related to other legislation. This first output of the group presents an overview of the different regulations involved in the management of biodiversity in the agricultural landscape. It aims to show possible gaps in linking of regulations, to present interim approaches already established in Member states to address these and to show an overview of implemented national strategies. It is also an invitation for other regulators to get in touch with this group and share their insights. [1] The members of this interest group thereby do not represent the view of their institutions and the information which is presented is neither binding for the corresponding institution nor the member states represented.

6.06.P-Tu304 What Happens When Everything Fails?

Alan Lawrence, Cambridge Environmental Assessments, United Kingdom

Registrations of plant protection products (PPPs) in the EU are facing pressure due to increased stringency applied to environmental risk assessments by Member State authorities and EFSA. This is already leading to the loss of active substances and products. The proposed consideration of spray calendars at the catchment and landscape scale would lead to further losses of PPPs. On the other hand, EU Regulation 1107/2009 highlights the importance of pesticides for agricultural production, and their continued availability to EU growers helps to prevent export of food production and according environmental disbenefits to other jurisdictions with less stringent authorisation procedures. Therefore, there is a need to find a way forward that ensures that EU growers have the tools required to produce reliable food while a desired level of environmental protection is achieved. Simply making pesticide risk assessments more challenging is not going to reverse declines of farmland wildlife in the EU. Here I outline how enhanced biodiversity at the landscape scale could offset apparent concerns regarding potentially adverse environmental effects of pesticides, providing the required tools for growers, while contributing to real biodiversity gains. The necessary trade-

offs between productivity and enhancement measures will be discussed, also the need to demonstrate environmental benefits beyond those provisioned by current agri-environmental and industry stewardship schemes, the scale of action required, and responsibilities. The aim is to foster discussion which may lead to constructive progress in this challenging area.

6.06.P-Tu305 ENTOSAFE - Edible Insects: From a Sustainable Food Production to a Food Safety Concern

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The use of insects as a food and feed source is nowadays considered a solution for the increasing food demands in the following years. With that in mind, the recently funded project “ENTOSAFE - Edible insects: From a sustainable food production to a food safety concern” aims to integrate the food safety and the environmental sustainability aspects of edible insect farming. ENTOSAFE will take advantage of a symbiotic multidisciplinary team with members from industry (EntoGreen® - Ingredient Odyssey, Portugal) and academia (Univ. of Aveiro and National Health Institute Doutor Ricardo Jorge, Portugal). The project is committed to provide knowledge on the accumulation and transfer of potential contaminants (e.g., metals, mycotoxins, PAHs, pharmaceuticals) in insects farmed in rearing facilities, contributing directly with data sets to the new legislation and regulatory limits, concerning the presence of these compounds in the substrate. ENTOSAFE brings new approaches, focusing on full bioaccumulation studies through a complete uptake phase, assessing the contaminant into insects in different times during this phase. Considering the lack of information on insects' elimination capacity to excrete contaminants from their body, an elimination phase will be studied in a non-contaminated substrate. ENTOSAFE is also promoting environmentally sustainable strategies while avoiding any wasted components from the process. The application of frass, as an organic fertilizer - produced by the digestion of the agricultural wastes or other residues by insects - on crops is presented as a promising solution to a zero-waste policy. Despite promising, the available information on the benefits that this by-product has on crop productivity is scarce. In this context, ENTOSAFE seeks evidence on the impact of this frass on agricultural soil's main functions and the key processes behind. The project will evaluate the effects in soil quality and functions, soil-biota interactions in soils enriched with organic amendments and plant responses to stressors, by performing a series of experiments from laboratory to greenhouse scale. In summary, ENTOSAFE will contribute to an upscaling on the use of edible insects as feed and food, covering not only safety concerns but also promoting the sustainability of insect rearing processes.

6.06.P-Tu306 Hazard Assessment of an Ento-Based Fertilizer Produced From Olive Pomace

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Olive pomace (OP) is a by-product of the olive oil industry and a problem in Mediterranean countries since it is considered highly toxic for both environment and human health. The use of insects to reduce and convert waste/residues into valuable products is growing as an emerging environmentally friendly solution, promoting a circular economy/agriculture and a zero-waste policy. Insects have the ability to convert and bio-transform different matrixes, producing valuable organic waste due to their metabolic activity, called frass, which consists of valuable biomass with high potential for agriculture (used as organic fertilizer). In this study, OP residues derived from a Portuguese olive oil production site have been used to feed black soldier fly (*H. illucens*) larvae. Thereafter, the obtained digested frass was firstly characterized chemically, looking mainly at phenolic compounds and metals - contaminants commonly present in OP residues. Later, frass was ecotoxicologically tested using standard model organisms (*Folsomia candida*, *Enchytraeus crypticus*), looking at mortality and reproduction using OECD standardized tests. Tests ran for 28 and 21 days, respectively, using soil amendments from 1% to 10% (w/w) ratios with OP frass mixed in Lufa 2.2 soil. As results demonstrated that frass did not affect the survival of soil organisms at the application rates tested, phytotoxicity assays were conducted evaluating the effects of frass on seed germination and the radicle growth of the seeds. For that, different monocotyledonous (e.g. *Lolium perenne*) and dicotyledonous (e.g. *Brassica oleracea*) plant seeds were used. A previously incubated frass with doses of 1%, 2.5%, 5% and 10% (w/w) ratios were added to Lufa 2.2 soil with 80% of the water holding capacity of the soil was conducted to produce testing extraction solutions (1/10 w/v distilled water). Test extraction solutions and the seeds were tested for 72h (28°C) in the darkness. Thereafter, the number of germinated seeds and the length of the roots were used to define the germination index determining whether the frass, as the soil additive, has a toxic effect. Also, to compare the effects of the frass as a soil amendment on the germination of the seeds, the same phytotoxicity experiment was conducted using mineral fertilizer (NPK) as a comparative soil amendment. These results are a screening approach to understand if frass produced from the bio digestion of OP may be used/tested in more complex scenarios.

6.07 Risk assessment, -management and -communication of environmental plastic pollution

6.07.T-01 Assessing Risks of Microplastics in Drinking Water to Inform Risk Communication and Management Strategies in California

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Recent findings of microplastics in drinking water prompted concern amongst scientists and policymakers due to the potential harm towards humans. In 2018, the California legislature passed Senate Bill 1422 requiring the California State Water Resources Control Board to (1) define microplastics, (2) develop a standardized method to monitor in drinking water and accredit laboratories, (3) monitor for microplastics in drinking water for four years, and (4) provide consumers with guidance regarding health impacts. Microplastics have been documented in drinking water, but their effects on human health from ingestion, or the concentrations at which those effects begin to manifest, are not established. To assess the available evidence for human health hazards of microplastics in drinking water, we convened an expert workshop, and conducted a systematic literature search. Studies were screened for quality criteria, including particle characterization, experimental design, and applicability for risk assessment prior to undergoing additional expert evaluation. Twelve mammalian toxicity studies were prioritized and subjected to qualitative evaluation by external experts, of which 7 reported adverse effects on male and female reproductive systems, while 5 reported effects on various other physiological endpoints. No single study met all desired quality criteria, but collectively toxic effects with respect to biomarkers of inflammation and oxidative stress represented a consistent trend. While it was possible to derive a conservative screening level to inform monitoring activities, it was not possible to extrapolate a human–health-based threshold value for microplastics, which is largely due to limited studies on polymers and shapes other than polystyrene spheres, uncertainties with measured apical endpoints, and missing exposure information. Findings from this assessment inform monitoring strategies for microplastics in California’s drinking water supplies. To aid consumers in interpreting detections of microplastics in drinking water, the expert workshop developed qualitative health-based guidance language.

6.07.T-02 Risk Assessment of Microplastics in Marine Ecosystems; From detection of Presence to Quantitative Assessment of Effects

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Conventional plastic objects do not decompose but they weather and fragment into increasingly small secondary microplastics ubiquitous in all marine environmental compartments, from arctic ice to deep sea sediments. Therefore, a quantitative assessment to know whether this presence reach levels that support ecological effects, and thus to quantify the risk posed by microplastics to marine ecosystems, is urgently needed. On the other hand, search for biodegradability have placed in the market compostable plastic materials. An ecotoxicological assessment of these alternatives is needed to see whether they reduce the potential impact compared to conventional plastics such as PE. Using standard marine models, we found no toxic effects of polyethylene (PE) bags, either conventional, recycled or oxodegradable. In contrast, all bags labelled as compostable tested showed a certain but moderate degree of toxicity on plankton organisms. Concerning expression of biomarker genes, no clear differences in the patterns of alteration were identified between both groups of bags. According to FTIR analysis the major component of the compostable bags were aliphatic-aromatic polyesters, most likely polybutylene-adipate-terephthalate, an oil-based polymer whose resin showed very low toxicity. According to GC-MS analysis, the qualitative composition of conventional vs. compostable additives was similar, including restricted low molecular weight phthalates such as DEHP and DBP. Therefore it can be concluded that replacement of PE by compostable plastic carrier bags do not represent any improvement but may even increase the hazard of these materials on the marine environment. On the other hand analyses of a microplastic aquatic toxicity database gathered from the literature supported that currently monitored plastic particle sizes pose a negligible risk to the global oceans, but techniques to assess smaller fractions are urgently needed to conduct ERA for those more hazardous sizes. In conclusion, replacement of polyethylene by compostable plastic materials do not warrant any improvement in the ecotoxicological hazard posed by plastics to the marine environment.

6.07.T-03 Policy Initiatives Targeting Plastic Pollution: Unfolding the Science Behind

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The intensive global plastic use and associated plastic pollution have caused concern for the potential risks to human health and the environment which. This has led to the adoption of numerous regulatory initiatives aiming to combat plastic pollution. Despite the considerable regulatory activity in the field of plastic, it appears that the actual risks of plastic to humans and the environment are still uncertain. This raises the question of to what extent the current plastic regulation is evidence-driven, a declared goal in the European Union. Therefore, the aim of this study was to investigate the scientific foundation for some of the key regulations targeting plastic pollution. Furthermore, we examined whether the knowledge base that the regulations rely on are considering the latest “state of the art” and discuss the different scientific foundations for guiding policy actions of environmental plastic pollution. Key regulations were selected based on both their importance with respect to regulation of plastics as well as their historical importance as drivers for societal actions on plastic pollution. We found that scientific evidence appears to be generally present in the scientific evidence for the policy initiatives analyzed in this study, and to a large extent for many initiatives. All the initiatives are supported by scientific articles and reports about among others plastic sources, ecological impacts of plastics and production and consumption patterns. Marine litter monitoring data was found to contribute to the evidence base for 4 out of the 6 policy initiatives and thereby appears to be one of the central scientific drivers behind the societal actions on plastic pollution. Other scientific tools applied when shaping the policy initiatives include risk assessment, impact assessment and life cycle

assessment. Despite the prevalent consideration and application of scientific evidence, there appears to be a broad recognition in the preparatory work of the initiatives that there is still a lot of uncertainty related to determining the harm of plastic pollution. In these cases, taking precautionary actions seems however to be justified, recalling not least the precautionary principle – a fundamental principle of European environmental regulation.

6.07.T-04 Examining Public Perspectives on Microplastic Pollution

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The topic of plastic pollution commonly encourages discussion regarding how environmental science is communicated. Public engagement with microplastic pollution has been significant and is arguably stronger than for other environmental issues such as climate change and ocean acidification. This has led to various hypotheses as to why this is and what social aspects may be influencing such trends. This study aimed to investigate public perceptions of microplastic pollution and to better understand how science communication could be improved to encourage greater public engagement with environmental protection strategies. A questionnaire assessing the perceived risk, control, progress and hope relating to microplastic pollution was developed in survey writing software Qualtrics. These themes of perception were based on previous related literature. The questionnaire also assessed environmental identity based on self reported factors of concern, emotional connection and consumer choice relating to the environment. In addition, self reported knowledge was recorded with a brief post-answer assessment to validate results. Both quantitative and qualitative responses were recorded. The questionnaire was distributed by third-party market research company PureProfile to collect as representative a sample of the Australian population as possible. Preliminary results revealed general public perceptions and misconceptions which highlighted a distance between the public and scientific perception of microplastic pollution. This calls attention to the effectiveness of environmental science communication itself, as well as to the challenges of adapting scientific findings for the wider media. The findings are of particular importance when considering the modern proliferation of misinformation and the effects of industry marketing. These factors are significantly affecting the perception of science communication and so need to be addressed with adapted strategies. To this end, interdisciplinary approaches to science communication are crucial for environmental scientists to be successful in their endeavours to promote public awareness and to support the uptake of effective environmental remediation strategies. To improve science communication will require a concerted effort between STEM (science, technology, engineering and mathematics) and HASS (humanities, arts and social sciences) disciplines to collaborate and communicate more effectively.

6.07.T-05 How Can We Test Plastic Pollution Perceptions and Behavior? A Feasibility Study With Danish Children Participating in "the Mass Experiment"

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Research suggests that behavior change programs can be fast and cost-effective solutions to plastic pollution alongside traditional environmental policy-making. Furthermore, encouraging change in perception and behaviour can be a tool to change consumption and waste handling towards increased circularity, which is of high concern in the EU. Beyond *knowledge*, predictors of pro-environmental behavior include *concern, social norms, nature-connectedness, identity* and *self-efficacy*. Citizen Science (CS) as a way to raise awareness and potentially change behavior show promise within plastic litter monitoring. We tested the feasibility of evaluating a nation-wide citizen science intervention, 'the Mass Experiment' (ME), with school students (age 7-16) in Denmark. With more than 57,000 students signed up for ME, this is to our knowledge one of the largest CS activity on plastic debris targeting young people. As an addition to the core CS activity we developed a voluntary and anonymous questionnaire to study the perceptions and behaviors of the students. We hypothesized that the intervention would increase *risk perception, self-efficacy* and *empowerment* as well as *self-reported actions*. Through 931 pre-surveys and 838 post-surveys aggregated at the team level (n=48), we found that the intervention had no significant overall effect on team, risk-perception, pro-environmental behaviors, nor self-efficacy or empowerment. However, unexpected patterns emerged for age effects, potentially advising some caution over the design of such CS activities particularly for younger children. We discuss methodological limitations, the high baseline for nearly all variables, the Danish context and the intervention itself and make recommendations for studying future CS interventions.

6.07 Risk assessment, -management and -communication of environmental plastic pollution (Virtual Only)

Presentation 6.07.V-01 was moved to session 4.15 (Virtual Only)

6.07 Risk assessment, -management and -communication of environmental plastic pollution (Poster)

6.07.P-We282 Flows of Seven Plastic Polymers Within Products in Norway From 2000 to 2050 As a Basis for LCA on Future Waste Management Practices and Recycling Targets

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Once considered a blessing for society, plastic is now increasingly being viewed as a burden to society. Production of plastic relies almost entirely on fossil fuels. In order to decrease dependence on fossil fuels and the associated emissions of greenhouse gases, the Norwegian government in their new plastic strategy calls for increased recycling of plastic. To achieve the Norwegian plastic strategy and increase recycling in Norway, the current and future flows of plastic need to be mapped out. Therefore, we

estimated the expected flows of plastic in Norwegian society from 2000 to 2050 of the seven most used polymers (low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polystyrene (PS), expanded polystyrene (EPS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET)) using a dynamic probabilistic material flow analysis (DPMFA). The model starts with the raw material, considers trade of the raw materials, unfinished and finished products and ends with the collection of recyclable material and waste and the subsequent waste treatment. In addition to the main categories of plastics, the model includes synthetic textile products and products related to fishing and maritime industry. Thus, the model considers the whole lifecycle of plastic and provides a comprehensive overview of the consumption of each polymer type. For the flows from 2000 to 2020, current data was used, while for the projection to 2050, a business-as-usual approach was applied assuming a linear trendline based on data from 2016 to 2020. Our model predicts the amount and polymer composition of future waste flows in Norway from production to waste. This research gives policy makers a basis for future waste management practices and increased recycling targets for plastic. It also allows waste collectors and processors to identify where in the value chain large amounts of high quality recyclable plastic products will become available for reuse and recycling.

6.07.P-We283 Will Increased Recycling of Plastic Also Increase the Exposure to Additives and Hazardous Chemicals Under Circular Economy? An Inventory of Chemical Additive in Plastic Products in Norway

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To achieve the European Green Deal objectives and facilitating the green transition, the Norwegian government set stringent targets to increase recycling rates. Regarding plastic products, at least 50% of plastic waste should be recycled in Norway from 2025 onward. Consequently, 50% of plastic products entering Norwegian market must be made of recycled plastic. However, the growing concern regarding the presence of hazardous chemicals in post-consumer plastic (PCP) products, poor quality of PCP, lack of knowledge on additives used in PCP and shortage in supply of PCP create massive challenge for plastic producers. This in turn, can counteract the objectives of some well-intended policies in increasing circularity by unintentionally increasing the recycling of hazardous chemicals in PCP and worsen the quality, which then shortens the lifespan of plastic products. Further, more additive chemicals must be added to PCP to improve their qualities for given applications, which will exacerbate the recycling of PCP and associated products under circular economy. In this work we established an inventory of chemical additives in consumer products. Using Norwegian status-quo data, we characterized the flows of additive chemicals within plastic products entering the Norwegian market and their subsequent flows to the waste stream. Plastic products are classified into 41 individual plastic products groups, within which the fraction of polymers was estimated. Several databases (e.g., ECHA, NORMAN) were used to quantify the presence of chemicals in plastic products. By coupling these data with waste treatment data at municipality level, we estimated the probability by which chemicals of concern can re-enter the market within recycled products. The results of this work can serve as a valuable tool for authorities in developing more effective targets for polymers and plastic products based on their potential chemical contents and applications.

6.07.P-We284 Reduction of Plastic Waste From Operation Rooms

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Plastic production and pollution continue to grow regardless of the well documented negative impacts on the environment. Healthcare facilities have shifted from multiple use products and tools to single use in recent years, accelerating the use of single use plastic products. This shift can be attributed to the low associated cost of plastic, sterile capabilities of plastic, and efficiency for the staff. The healthcare industry is responsible for emitting copious amounts of CO₂ globally due to resource intensive procedures and facilities. Currently there is a gap of knowledge when accounting for the specific types and amounts of plastic products and packaging being used in the operation rooms (ORs). This project aims to analyze how a reduction in the amount of plastic waste in ORs can be achieved by facilitating a transition towards a more circular economy of products used in the operation rooms. To do this, identification, quantification and polymer analysis of relevant plastic products and packaging was performed at ORs in two Danish hospitals (Næstved Sygehus and Slagelse Sygehus) in Region Zealand, Denmark. Direct observations of surgeries within both hospitals were conducted, FTIR polymer analysis of the identified products and packaging was conducted, and key informant surveys were conducted including nurses, doctors, and anesthesiologists of both hospitals. The direct interaction of the staff with these products makes their opinions highly valuable, it enables an analysis of perceived barriers for the circular transition. Preliminary results suggest that there are slight differences of plastic waste generation between hospitals. The observed deviations in plastic use are suggestive to differences in procedure of the different surgical departments. Results also suggest that the most prominent type of plastic waste found in both hospitals was composite plastic and paper packaging. Up to 38% of plastic waste in Slagelse Sygehus operation rooms, and up to 26% in Næstved Sygehus. However, product design of the composites makes recycling them currently impossible in procedure, according to staff. Further analysis will suggest products for redesign, recycle, or reuse, to reduce overall plastic waste. The current value chain of medical plastic products has mostly followed a linear value chain which continues to exacerbate the plastic crisis. By including more circularity among products, fractions of medical plastic waste can be mitigated.

6.07.P-We285 Microplastics in Agricultural Soil - Experiences of Agricultural Plastics Users

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Various plastic products are used in horticulture and agriculture. For example, plastic can protect, control and accelerate growth of plants, and it can be used to transport and store products. Despite numerous benefits, agricultural plastics are also potential

sources of microplastics to soil. The MicrAgri project, funded by the Ministry of Agriculture and Forestry of Finland by Makera Fund, identifies the main sources of emissions of microplastics to agricultural land in Finland, assesses the effects of plastic particles in the soil and proposes measures to reduce microplastics emissions in agriculture and horticulture. This presentation shows the results of an open online survey conducted within the framework of the project during the fall of 2021. The survey examines the agricultural plastics used by agricultural and horticultural producers, landscapers and home gardeners, users' remarks on degradation of conventional and biodegradable plastics into plastic particles and suggestions on how to prevent the release of plastics into the environment. The survey also draws respondents' attention to the plastic challenges in agriculture, provides information on the subject and on the other hand helps to find out what kind of information on plastics is needed by the agricultural and horticultural producers, landscapers and home gardeners.

6.07.P-We286 Human Risk Assessment of Organophosphate Ester Plasticizers in Drinking Water and Other Beverages

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According to the latest available data, plastic production has reached almost 370 million tons worldwide, which 58 million tons correspond to Europe. Packaging is the main application for plastics, mostly for food and beverages. Plastic materials contains chemical additives, which may release to the environment during its use and manufacturing, and also may migrate from the package to the contained food. This issue is an associated outcome of plastic pollution, and it is a human exposition path for organic and inorganic contaminants that must be explored. Organophosphate esters (OPEs) are a group of chemicals commonly used as plastic additives and flame retardants. Their reported toxic effects, such as the development of asthma and allergies, has led to an increasing concern for these emerging pollutants. Tri-n-butyl phosphate (TNBP) has been shown to be an endocrine disruptor. In fact, human exposure to TNBP, 2-ethylhexyldiphenyl phosphate (EHDPP) or triphenyl phosphate (TPHP) seems to be associated with cervical cancer. Additionally, the USEPA have recently established oral reference doses (RfD) and oral cancer slope factors (SFO) for some of the OPEs. Based on these values, an assessment of the non-carcinogenic (non-CR) and carcinogenic (CR) risks of human exposure to OPEs can be performed. Multiple studies about OPE occurrence in air or ground water have been done. Nonetheless, there is a lack of data about OPE exposure through beverages or food ingestion. The main objective of the present study is to evaluate OPE occurrence in different beverage samples, such as tap water, bottled water, cola drinks, juices, wines, tea and coffee infusions. Likewise, an assessment of human risk impact will be carried out. OPEs were detected in most of the analysed samples. The highest levels were found in cola drinks, with a mean value of 2906 ng/L. EHDPP was the major contributor, with ranges between 29.3 and 5855 ng/L. Taking into account these results, it is possible to accomplish an assessment of estimated daily intakes (EDIs) related to water and beverages ingestion. Based on Spanish *per capita* consumption, the highest values for EDI in adults were obtained for EHDPP in cola drinks, with 2.18 ng/kg body weight/day. These results showed up the human exposure to plastic additives in drinks and, hence, the importance of focusing on chemicals associated to plastic pollution, in order to contribute to the development of legislation to restrict the use of plastics.

6.07.P-We287 Methodological Approach to Assess Measures to Reduce Microplastics in the Environment

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Microplastics are found in nearly every compartment of the environment: air, urban, rural and agriculture soil, sediments, sea, surface and river water, shorelines, even in the arctic, in animals and the humans. Hence, the topic of microplastics is getting increasingly more attention from research community. Although there are ongoing efforts to increase our understanding about microplastics, there are still some important research gaps. One of which is connecting the knowledge from experimental research to propose mitigation measures. The present work intends to tackle the microplastics issue from broader perspective. The aim of this research is to develop robust proposals on how to assess and to mitigate the impacts of microplastics. In order to do that the researcher investigates the point and quantities of microplastics losses along the whole life cycle of selected products / applications. This is combined with a subsequential fate modelling to understand the pathways of microplastics from point of loss to environmental compartments and to estimate the amounts that reach final environmental compartments. In order to understand the effectiveness of measures (e. g. restriction, ban, information education), various intervention scenarios will be developed. These scenarios will be evaluated by prospective life cycle assessments including a new impact assessment methodology, which addresses the plastics persistence in the environment. It is anticipated that the researcher comes up with a ranking of possible behavior and policy recommendations to mitigate microplastics' impacts. The developed methodology will be applied to three field of plastic application: tires, textiles and construction insulation material (expanded polystyrene). Main reason for selecting these microplastics sources is that to look into different point of loss and pathways to environmental compartments, as well as difference in material properties (in particular density, shape and size). Although the research focuses on certain microplastics sources as a case study, it is expected that the methodology developed and used can be also adapted to other microplastics sources.

6.07.P-We288 How Environmental Regulation Can Drive Innovation - Examination of Three EU Environmental Regulations

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Regulation is often seen as a barrier to innovation. However, if done properly, it can serve as a driver of innovation. To understand how environmental regulation can be designed to stimulate innovation, we scrutinise the scientific literature related to

regulation, innovation and the environment. Fifty carefully selected studies are examined with regard to their scope, results and geographical affiliation, and their findings are distilled into ten lessons on how to design environmental regulation to stimulate innovation: 1) Environmental regulation should guide innovation; 2) The transition period is vital for industry if it wishes to adapt and find innovative solutions; 3) Clear and concise regulations; 4) Flexible regulation and compliance deadlines; 5) Provide economic incentives and other benefits for (doing more than) complying; 6) A regulatory process where all voices are heard; 7) Evaluation of effects on innovation; 8) Secure support and capacity-building; 9) Multiple regulatory measures are needed; 10) Minimise the costs of compliance: the proportionality principle. Subsequently, we analyse three recent EU environmental regulatory initiatives (Nano-specific REACH annex revisions, the Single-Use Plastic Directive and the Waste Framework Directive) and evaluate them against identified lessons learned. In conclusion, the three regulations do support most of the lessons learned; however, none of them supports economic incentives for doing more than complying, meaning that regulatory-driven innovation is at a risk of stalling or coming to a halt when compliance requirements are met.

6.07.P-We289 The European Union Against Plastic Pollution: How to Shift From a Fragmented to a Cohesive Legal Framework?

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Since the beginning of mass production, plastic items have been crucial in our daily lives. Thanks to their physical and chemical properties, plastic materials have proven almost irreplaceable in a number of economic sectors: packaging, automotive, building and construction, textile and many others. At the same time, the “overaccumulation” of plastics in the environment, and its adverse effects on habitats, wildlife and (potentially) human health, represent a call for action to decision-makers around the globe. The situation in the EU27 Member States is not dissimilar: almost 26 million tonnes of plastic waste is generated herein every year, whose 24,9% is still destined to landfill. Thanks to the new *Circular Economy Action Plan*, approved in March 2020 by the Commission, EU countries are slowly but steadily shifting to a carbon neutral, circular economy, in the attempt to reduce the pressure on natural resources and, parallelly, facilitate sustainable economic growth. The EU’s *Plastic Strategy* is a crucial part of such vision, and it is promising to change the way plastic is designed, produced, used and treated after consumption. Positive effects of the Strategy include: a more effective protection of our environment, especially the marine one, the reduction of greenhouse gas emissions, a reduced need for imported fossil energy sources, more sustainable production and consumption patterns. As promising as it may sound, the road ahead is still long. From a regulatory perspective, the design of new legal instruments, the amendment of existing ones, and the coordination among the several relevant policy areas, would require a considerable effort. An analysis of the current European Union legal framework on plastic, inclusive of both binding and voluntary instruments, could serve to detect blind spots in the current governance and move toward an improved plastic management in the EU.

6.07.P-We290 Are Europeans Worried About Microplastic Pollution? Attitudes, Actions, and Factors Behind

Renata Dagiliute and Aušra Dikšaitytė, Vytautas Magnus University, Lithuania

Microplastic pollution attracts research and policy attention due to its negative human health and environmental impacts. Though many of microplastics is intentionally added into the products, the majority of the microplastic particles found in the environment is a result of use and pollution of bigger plastic units. The study, referring to the Eurobarometer survey on environmental attitudes (2020), aims to analyse the trends in attitudes toward marine pollution, microplastic pollution, behavioural response and factors influencing attitudes and actions taken regarding plastic and microplastic pollution reduction. Results show that in general marine pollution is considered as important environmental issue only by 36.5% Europeans. However, 49% respondents totally agree and 38.6% tend to agree that they are worried about microplastic pollution. The most concerned are citizens of Cyprus and Spain. Some 52.4% Europeans indicated that protection of the environment is personally very important to them. Regarding the actions related to the plastic and microplastic pollution, respondents indicated to avoid of single use plastic products (except plastic bags) (43.8%) and overpacked products (28.4%), and most often Europeans separated waste (65.5%). Regression analyses indicate that those considering marine pollution as important environmental problem and environmental protection being an important thing personally more often are worried about microplastic pollution ($p < 0.05$). In turn, those from small households, with higher incomes, women, older and from the bigger towns as well as those thinking that marine pollution is an important problem and considering environmental protection personally very important take more actions to reduce environmental pollution in general ($p < 0.05$). The most important in reducing plastic waste and littering for the Europeans appeared to be the role of industries and retailers, as well as product design (66.3%), extra charges for single use plastic products are acknowledged as very important measure only by 33.3% respondents. The main source of environmental information remains traditional channels: TV and newspapers, but internet and social media is gaining attention. In-between social media channels, Facebook is the leading source of the environmental information for respondents.

6.08 Tackling Marine Pollution on the Road to Sustainable Use of Marine Ecosystem (Virtual Only)

6.08.V-01 From Past to Future - Longterm Trends of Marine Pollution in the German Bight of the North Sea

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Pollution has been an environmental issue for decades and great efforts have been made to monitor, identify and survey contaminants of concern over a number of years. The main objective of the European Marine Strategy Framework Directive is to achieve “Good Environmental Status” of EU waters by 2020. However, this goal was not yet met for the German North Sea, also

due to increased concentrations of contaminants. This highlights the need for a better understanding of pollution as a stressor in general and calling for targeted action in order to improve ecosystem health. The Federal Maritime and Hydrography Agency has monitored contaminants in the German EEZ for the past decades ranging back to the mid 70ies for selected substances and sampling sites. Our contaminant monitoring efforts focus on heavy metals and a wide range of organic compounds (e.g., organochlorines, polycyclic aromatic hydrocarbons and pesticides). This data was analyzed for two different time periods (1980 – 2000; 2001 – 2020) to evaluate changes over time. Here, we present long-term trends for several contaminants of concern such as heavy metals and organic compounds in the sediment and water column. Furthermore, we compared the long-term changes in the spatial distribution of selected contaminants between the two periods to identify potential sites of elevated pollution (“Hot-spots” of pollution). This analysis forms the basis for multiple stressor modeling approaches for future changes and management scenarios that will be conducted in the wide spanning project MuSSeL (Multiple Stressors on North Sea life). Our findings will aid future monitoring efforts and help make informed decisions for managing the marine environment.

6.08.V-02 Global Governance in Arctic Waters - New Times. New Stressors. Catching up With Pharmaceuticals

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Arctic ecosystems are increasingly under pressure, not only from climatic stressors, resource extraction, and long-range transport of Persistent Organic Pollutants (POPs), but also from an increased use and release of Pharmaceuticals and Personal Care Products (PPCP). In Svalbard, an archipelago under Norwegian sovereignty in the High North, urbanization and expanded tourism has exacerbating the issue of PPCPs accumulation in the region. The primary source of its release into aquatic ecosystems stemming from untreated sewage and lack of Wastewater Treatment Plants (WWTPs). This study applies the research surrounding sources of hazardous bioaccumulation and discusses mitigation alternatives for PPCPs within a governance framework since today, few regulations regarding human waste disposal are enforced in the Arctic. We held in-depth interviews and a participatory stakeholder workshop in Longyearbyen and Ny-Ålesund in 2021 to learn from experts about their perceptions of challenges, opportunities and synergies in terms of PPCP governance in Svalbard. This study found that overall, governance is severely lacking at all levels of analysis, from local to global, to prevent the harmful release of PPCPs in the waters and environment surrounding Svalbard. An inclusive approach with co-production of policy solution options is necessary to find a suite of solutions that will ensure that this new emerging environmental threat is handled so that the Arctic biodiversity is protected against it, and the local community of Longyearbyen may be a future good example of this for further research.

6.08.V-03 Heavy Metal Exposure in Bottlenose Dolphins (*Tursiops truncatus*): Differences in Blood Concentrations of Hg, Cd, Pb and As Between Captive and Wild Dolphins

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Bottlenose dolphin as a top predator species may serve as a good monitor of contaminants in marine ecosystem. Heavy metals concentrations in blood of 30 bottlenose dolphins (*Tursiops truncatus*) have been investigated in this study. Two groups of animals have been considered: captive dolphins under human care (n=14, group 1) and wild dolphins (n=16, group 2). Group 1 was composed of 14 captive dolphins (4 males and 10 females) from two specialized centers in Valencia (Spain). Group 2 was composed of 16 wild dolphins inhabitants of the Sarasota Bay in the context of Sarasota Dolphin Research Program carried out in Sarasota (Florida, USA). Veterinarians participated in the health control of the animals and no dolphin presented lesions or active health concerns during the sampling. As a part of the health assessment, citrated and heparinized whole blood samples were collected. Finally, the animals were released to the sea. Dolphin samples were collected under National Marine Fisheries Service Scientific Research Permit No. 20455, and it was approved by the Mote Marine Laboratory Institutional Animal Care and Use Committee (IACUC). For analysis of Pb, Cd and As blood samples were digested using an Ultrawave system (Milestone, Inc. USA). Metal concentrations were analysed by ICP-MS. Analysis of Hg were carried out with a direct mercury analyser (DMA80, Milestone, USA). Hg showed the highest concentration, followed by As, Pb and Cd. Significant differences were observed when the blood metal levels were compared between captive and wild dolphins. Concentrations of mercury, arsenic and cadmium in wild dolphins were higher than in captive dolphins (Hg: 387.5±236.8 ng/g vs 25.5±6.9 ng/g; As: 46.1±40.68 vs 17.39±9.12 ng/g; Cd: 10.18±22.9 ng/g vs non-detected). On the contrary, in the case of lead, mean concentration was lower in wild (23.4±51.0) than in captive (44.3±35.6 ng/g) dolphins. No significant differences in metal concentrations were found between sexes. Correlations with age and weight of the dolphins were evaluated with the blood metal concentrations. A negative correlation was found between As concentration and the weight of the dolphin (-0.661, p=0.01), but only in captive dolphins. On the other hand positive correlations were found between mercury concentrations of wild dolphins with the age (0.775, p< 0.01) and the weight (0.736, p< 0.01, n=14), respectively. Between metal concentrations only a high correlation cadmium-lead was found (0.961, p< 0.001).

6.08.V-04 Integrating Ecotoxicology Into Decommissioning Offshore Oil and Gas Infrastructure Decision Making

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There has been a substantial body of work investigating the benefits of leaving offshore oil and gas infrastructure *in-situ* as part of decommissioning operations, such as the provision of artificial reefs that increase marine organism biomass, biodiversity and potentially support fisheries. Such research has been conducted in Europe, the US and the Asia Pacific. However, there is no

framework to address potential contaminants within the decommissioned infrastructure (e.g. scale and associated bound contaminants on internal surfaces of production pipelines) and even less is known about the long-term environmental fate of associated contaminants. Consequently, there is little publicly available information on contaminant assessments for offshore infrastructure. Although contaminants are initially contained in the infrastructure, corrosion may lead to them being released to the surrounding environment, where bioaccumulation and subsequent ecotoxicological effects could occur. Current assessments of infrastructure-associated contaminants rely on model predictions with untested assumptions, therefore model validation and supporting data are urgently required to inform risk assessments. Given that governments and industry around the world have a significant future liability in ensuring safe decommissioning of offshore infrastructure, successful decommissioning should maximize environmental benefits and cultural and economic value, while minimising the costs and risk of the activity. SETAC members represent a range of disciplines (ecology, chemistry, ecotoxicology, risk assessments etc) required to address critical data gaps about contaminants in offshore oil and gas infrastructure. We propose the formation of a SETAC interest group (IG) to leverage the expertise in our society and to facilitate knowledge exchange needed to maximise environmental benefit and inform key decisions around decommissioning. This poster will present an overview of the offshore decommissioning process and highlight the need to integrate ecotoxicology, facilitated by a new SETAC IG, into decommissioning policy frameworks.

6.08.V-05 Produced Water Discharge and Impact on Marine Environment

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The discharge of produced water is of concern in view of potential environmental impact. Approach involving mere chemical analysis is no longer considered sufficient. A holistic approach entailing whole effluent toxicity (WET) assessment from an ecotoxicological perspective has been increasingly advocated. Moreover, there is a need to employ a risk-based approach/assessment (RBA). RBA has been instigated under OSPAR initiative and member countries have put in place appropriate implementation programmes. This presentation provides a comprehensive perspective of the UK RBA process. An insight into the development of the initial trial to the formulation of the current stepwise programme process involving chemical analysis, ecotoxicity testing, modelling and reporting. Key considerations of the produced water risk management elements are discussed. The aspect of intrinsic toxicity contribution from production chemicals and naturally occurring substances is examined. Essential considerations of the key regulatory criteria and drivers are analysed. The implication of the findings of the initial phase of the UK risk-based approach (RBA) programme are highlighted and how these impact future sustainable developments. A definitive evaluation of the global RBA panorama is presented to rationalise top-down and bottom-up strategies conveying the global disparity that exists in the regulatory approach and existing infrastructure to consolidate the way forward. The predictions are that major oil companies not only will continue with current level of production but will expand their operations that extract and refine fossil fuels. The realisation is becoming all too apparent that in order to have a stable and safe planet reaching net zero emission is imperative. Whilst renewables are viewed favourable in terms of significant growth area, indubitably the issues and considerations pertaining to safe discharge of produced water remain at the forefront. The intrinsic elements of importance for evaluation including production chemicals and naturally occurring substances play a key part. Methodology for assessment and inference from a global regulatory perspective lacks definitive approach and the disparity that exists inevitably compounds issues and instils lack of confidence. Scope for pragmatic and prudent solutions to address pertinent issues will be highlighted. **RBA Toxicity Marine Ecosystem**

6.08 Tackling Marine Pollution on the Road to Sustainable Use of Marine Ecosystem (Poster)

6.08.P-Mo263 Acute Toxicity of Arsenic-Based Chemical Warfare Agent Clark I to Aquatic Primary Producers

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Sea-dumping of Chemical Warfare Agents (CWAs) was a common practice worldwide during the 20th century. CWAs dumped in the seas and freshwaters pose a potential environmental hazard. The greatest concerns are raised by the presence of submerged arsenic-based CWAs, due to their persistence in aquatic environment and the fact that after degradation in water they may be a source of secondary pollution and release of toxic inorganic arsenic. The ecological effects of these CWAs on aquatic primary producers have not been studied yet. In standardized laboratory bioassays we quantified the inhibitory effects of Clark I (diphenylchloroarsine) – an incapacitating agent, which was used in combat during World War I and after world wars as a riot control agent, on population growth rates of three species of green-algae (*Scenedesmus obliquus*, *Chlorella vulgaris*, *Chlamydomonas klinobasis*) and three species of cyanobacteria (*Synechococcus elongatus*, *Anabaena cylindrica* and *Aphanizomenon gracile*). Clark I was found to be very toxic to all of the tested species of primary producers. The species differed from each other in susceptibility to the presence of Clark I, the variation among species in LOEC spanned the range of three orders of magnitude. Green-algae on average seemed to be less susceptible than cyanobacteria. The effects of Clark I on aquatic primary producers should be taken into account in assessments of the impact of organic-arsenic CWAs on aquatic ecosystems.

6.08.P-Mo264 Acute Toxicity of Arsenic-Based Chemical Warfare Agents to Danio rerio Embryos

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During the 20th century, several hundreds of thousands tons of munitions containing chemical warfare agents (CWAs) were dumped in the seas and oceans. Recently, CWAs have started to leak from the corroded munitions and their concentrations in the bottom waters are expected to peak in the next decades. Based on the physical and chemical parameters of sea-dumped CWA and their effects on aquatic invertebrates, it has been established, that among CWA, arsenic-based agents, and their degradation products are the most threatening chemical hazards with long lasting effects on aquatic ecosystems. However, as of yet, there is still a lack of knowledge about the toxicity of these arsenic-based CWA on aquatic vertebrates, such as fish, which are not only crucial for the marine ecosystems but also for human economy. There is a lot of ambiguity regarding the toxicity of CWA to marine life, as existing studies do not comply to full track of OECD (Organisation for Economic Cooperation and Development) toxicity establishment procedure. The aim of this study was to address this gap, by providing real-data quantification of arsenic based CWAs toxicity to the embryos of fish (using a model species, *Danio rerio*), which is essential for further environmental risk assessment. In order to estimate the toxicity thresholds (NOEC, LC50) of arsenic-based CWAs (Clark I, Adamsite, phenyldichloroarsine (PDCA)), a CWA-related compound (triphenylarsine - TPA), as well as two arsenic-based CWA degradation products (Adamsite[ox] and TPA[ox]), standardized assays were performed, following OECD guidelines (Fish Embryo Acute Toxicity Test, no. 236). The tested concentrations of arsenic-based CWA caused lethal effects to *Danio rerio* embryos either after 24, 48, 72 or 96h exposure. However, the tested CWA-related compound (TPA) showed no adverse effects even at maximum solubility and the CWA degradation products have shown no adverse effects at concentrations up to 100 mg × L⁻¹.

6.08.P-Mo265 Plastic Pollution in the South Eastern Pacific Region: Identifying "Hot-Spots" to Inform Sustainable Solutions

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Plastic pollution is a well-known and persistent threat in oceanic environments worldwide. In the South Eastern (SE) Pacific, ocean plastic pollution is particularly concerning due to the region's unique habitats and exceptional biodiversity. The coastal communities here are highly dependent on the marine environment for their livelihoods, sustenance, and wellbeing (e.g. tourism or fishing). It is therefore imperative to understand the sources and extent of plastic contamination in the area to help build local and sustainable solutions. Thus, to better understand the extent of plastic contamination in the region and help to find solutions for this emerging issue, we conducted a meta-analysis of peer-reviewed literature on macro- and microplastic debris in the ocean and coastal beaches of nine countries in the SE Pacific region (Mexico, El Salvador, Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Peru and Chile). We combined quantitative abundance data on macro- and microplastics with unpublished results from two partners of this project: Ichthion (Ecuador) and Pro Delphinus (Peru). The present study had three main aims: (1) assess the status of plastic contamination in the SE Pacific region, (2) overlay the identified "hot-spots" of plastic contamination with Marine Protected Areas (MPAs) and (3) provide solutions and mitigation actions based on economics and societal change. Our analysis revealed that over 8 years of research, a considerably low number of studies on plastic pollution have been published, especially for some countries such as Nicaragua, Panama, El Salvador, Colombia and Peru. There is also a significant data gap for microplastic assessment in the region, mainly due to technical limitations and lack of resources. Nevertheless, the current data suggests that many of the MPAs in the SE Pacific may be affected by plastic, requiring urgent action. The results of this study will hopefully help to build and promote solutions informed by robust science, and influence policy makers in future decisions.

6.08.P-Mo266 Effects of Plastic Associated Chemicals on the Development and Settlement of Early Life Stages of Four Common Coral Reef Invertebrates

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Plastic materials are currently recognized as an emerging issue of international concern threatening marine environments worldwide. Plastic additives (PAs) are chemical compounds incorporated into plastics during production, many of these chemicals are known endocrine disruptors and can potentially leach out of plastic debris into the environment. In the marine environment, biota-debris interactions are more likely to occur in coastal marine habitats featuring high biological activity such as fringing coral reefs. While coral reefs provide important ecosystem services that benefit society, our knowledge of plastic pollution impact on coral-reef organisms is still scarce, and no data exist on PAs effect on key reef-building species. As reef population structure is directly linked to reproduction and offspring settlement processes, the endocrine disruptor properties of PAs can play an important role in a coral reef community structure, even more so, if these effects differ among species. This study examines the effects of exposure to environmental and high concentrations of dibutyl phthalate (DBP), dimethyl phthalate, (DMP), 4-nonylphenol (4-NP), and bisphenol A (BPA), on early life stages of four coral reef invertebrates common in the Red Sea. We measured the following parameters: (1) Larvae settlement of the solitary ascidian *Herdmania momus*, the soft coral *Rhytisma fulvum fulvum*, and the stony coral *Stylophora pistillata*, (2) Fertilization success, and larvae development of *Herdmania momus*, and the calcifying hydrozoan *Millepora dichotoma*. All applied PAs affected negatively and unevenly the success of the development or settlement of one or more of the tested species, where the high concentration of 4-NP had the largest effect on all species. Although environmental concentrations of PAs found in seawaters are far below the high concentrations used in our experiments, it is the level of PAs within the body of the organism that the gametes and brooding planulae are exposed to. Each of the chosen organisms represents a major taxonomical group that can be found in tropical coral reefs around the world. Our findings

demonstrate for the first time the negative effects of PAs on coral-reef organisms, and specifically, the significant effect found during exposure to environmental concentrations of DBP and 4-NP on reef-building corals. This study is an alarm call for future investigation on the effects of PAs on coral reef organisms.

6.08.P-Mo267 Citizen Observation of Plastic Pollution in African Coastal Ecosystems to Address Data Gaps in Marine Litter Distribution

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The accumulation of plastic litter in coastal environments has become an issue of high priority for policymakers around the globe, due to the potential hazardous effects to biota and human health, and the impact on ecosystem services and in local economies. To develop effective mitigation measures, it is critical to acquire knowledge on the distribution and levels of plastic litter. However, in many regions, such as West Africa, the exact quantity of plastics reaching coastal areas is still poorly known. To address the data gaps in marine plastic litter distribution worldwide, citizen science programs are instrumental in complementing shoreline assessments, and are effective in increasing public awareness of plastic pollution. The Citizen Observation of Local Litter in coastal ECosysTems (COLLECT) project is a citizen science initiative which aims to acquire distribution and abundance data of coastal plastic debris in seven countries, in Africa (Benin, Cabo Verde, Côte d'Ivoire, Ghana, Morocco, Nigeria) and Asia (Malaysia). The project consists of training local students (15-18 years old) from secondary cycle institutions on sampling and analysing macro, meso and microplastic in beach sediments, using scientific procedures. The project will also measure the impact of the citizen science intervention by assessing shifts in ocean literacy and pro-environmental behaviour, while simultaneously considering gender differences. The COLLECT project contributes to the United Nations' Sustainable Development Goals by focusing on #11 the sustainability of communities and #14 the sustainable use of the ocean. Besides, the project relates to #3 the impact on good health and wellbeing and #5 gender equality, while #12 promoting a responsible disposal of consumer goods. COLLECT also reaches to the UN Ocean Decade challenges #1 understanding and beating marine pollution, #9 skills, knowledge and technology for all, and #10 change humanity's relationship with the ocean. The results from COLLECT will contribute to establishing baseline information on coastal plastic debris, with citizen science being an enabler of open science, allowing data to be freely available to the public, academics and policymakers. Results will further contribute to the identification of hotspots of plastic coastal litter, and bring awareness to local communities on the potential consequences of plastic pollution.

6.08.P-Mo268 Integrated Assessment of Chemical Contamination and Its Biological Effects Through Monitoring of Sediment, Mussels and Flatfishes

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A program to monitor chemical contamination and its effects in the marine environment (SELI) has been implemented in France within the European Marine Strategy Framework Directive context. Campaigns organized around estuaries of major French rivers, Seine and Loire, have provided measurements of various ecotoxicological parameters including contaminant concentrations in three matrices (sediment, mussels and soles) and biomarkers (in mussels and soles). The large number of these parameters complicates the overall ecotoxicological assessment and the comparison of different marine areas. The integration of the parameters in the form of synthetic indicators enables the information to be condensed in a comprehensible manner. Two approaches based on the comparison of parameters to thresholds were used. The first one counts the threshold exceedances of the parameters (derived from ICES 2012 method) while the other one calculates an average ratio of the values of these parameters on their environmental threshold (derived from HELCOM CHASE method). Graphical representations of the results and the use of quality classes allow for a quick visualization of the impacts of contamination over several sites. Between Loire and Seine, despite different levels of contamination, preliminary results suggested that biological effects were similar. This integrated multi-matrix approach, chemical and biological, could be extended to other marine regions to monitor the evolution of marine ecosystems with respect to chemical contamination.

6.08.P-Mo269 Critical Review of the Risk Based Approach for Produced Water Discharges From Offshore Oil Production

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Produced water constitutes the largest wastewater stream associated with offshore oil extraction. In 2018 the estimated discharge from offshore activities in the North East Atlantic was 300 million m³. Produced water is a complex mixture of chemicals potentially causing adverse effects on the receiving marine environment if not managed properly. The compounds of specific concern includes metals, benzene, toluene, ethylbenzene and xylene, polycyclic aromatic hydrocarbons, phenol, alkyl phenol,

organic acids and naturally occurring radioactive materials and production chemicals (e.g. biocides). The discharge must comply with governing regulations of the respective area. Common limit values are defined by the Oil in Water (OiW) content by either a daily maximum of 20 to 100 mg/L or a monthly average ranging from 29 to 40 mg/L. Often the OiW criteria is fulfilled as an example the reported annual average since 2010 for the Danish Underground Consortium was 7 mg/L. However, there is concern of adverse effects related to the aqueous fraction independent of the OiW content. Consequently, produced water discharges in the North East Atlantic has since 2010, been managed using a Risk Based Approach (RBA) considering the risk of the whole discharge in relation to the sensitivity of the receiving environment. The RBA consists of six steps, 1) Data collection, 2) Hazard Assessment, 3) Exposure Assessment, 4) Risk Characterization, 5) Risk Management and 6) Monitoring. Inherently, complex mixtures such as produced water are difficult to characterize both in terms of chemical constituents but also in terms of behavior in standardized test setups. Consequently, data curation and reporting criteria is key to avoid introduction of unnecessary uncertainties carrying over and amplifying in the following steps of the RBA. This poster presents the results of a literature review considering the regulatory adequacy of the data available in the scientific literature for use in the RBA. Additionally, the poster highlights limitations to the current iteration of the RBA and propose recommendations to improve the RBA for a more transparent evaluation of the risk associated with the produced water discharge.

6.08.P-Mo270 Effects of Copper and 4-Tert-Octylphenol in Acute and Chronic Exposure on Biomarkers in Amphipod Crustaceans (Baltic Sea)

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Alkyl-phenols, the group of nonionic surfactants, are industrial pollutants commonly found in wastewater. Octylphenol, one of the most toxic compounds to aquatic organisms, is not easily degraded in the environment, and has the potential to cause significant endocrine disruption effects. Copper is among the main active substances contained in antifouling paints on boats used for many years in Europe. Amphipods are important components of aquatic ecosystems and nowadays have been widely used to assess environmental pollution. This study aimed to determine potential risks of copper < st1:citation w:st="on">(Cu) and 4-tert-octylphenol < st1:citation w:st="on">(4t-OP) at acute and chronic exposure on various amphipod species and assess their health status by responses of different biomarkers < st1:citation w:st="on">(oxidative stress, reproductive health and others). The acute < st1:citation w:st="on">(96-hour) or chronic < st1:citation w:st="on">(42 Days) toxicity of copper and 4-tert-octylphenol were detected using different amphipod species based on ISO standards < st1:citation w:st="on">(16712:2005 and 16303:2013). Selected biomarkers (AChE, GST, CAT and reproductive disorders) were measured at the end of acute and chronic tests to represent different types of biological responses. The sublethal concentrations < st1:citation w:st="on">(LC₅₀) varied from 0.2 mg/l to more than 10 mg/l after 96-h exposure of Cu and were higher than 0.5 mg/l after 96-h exposure of 4t-OP. The chronic toxicity of 4t-OP was more pronounced to native species: *Monoporeia affinis* (LC₅₀ 0.65 µg/L) and *Gammarus oceanicus* < st1:citation w:st="on">(5 µg/L) than laboratory cultivated *Hyalella azteca* < st1:citation w:st="on">(294 µg/L, respectively). Enzymatic biomarkers showed significant (ANOVA p< 0.05) influence of chemicals on organisms at elevated concentrations compared to control. The reproductive potential < st1:citation w:st="on">(fecundity) in *H. azteca* averaging 5.7 eggs/per female and body size of organisms after 42 days exposure to 4t-OP did not show significant (ANOVA p>0.05) differences in *H. azteca* development compared to control. However, high reproductive disorders as malformed embryos per females (1.3-10%), as well as undeveloped and dead eggs < st1:citation w:st="on">(5-25%) were detected in higher 4t-OP concentrations. Using another species, *Gmelinoides fasciatus*, high mortality of embryos and number of them that stopped development < st1:citation w:st="on">(>50%) were identified after chronic exposure to 0.5 µg/L of 4t-OP. In general, Cu and 4t-OP concentrations of ecotoxicity tests and biomarker responses indicate the negative effects on amphipods' health or reproductive quality in long-term exposure. Research is supported by ERDF post-doctoral research grant 1.1.1.2/16/I/001 < st1:citation w:st="on">(application No 1.1.1.2/VIAA/3/19/465).

6.08.P-Mo271 Preliminary Insights on Sea Surface Currents and Primary Production Roles to the Surface Distribution of Polycyclic Aromatic Hydrocarbons at the Southern Okhotsk Sea

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Southern Okhotsk Sea is a fishing ground for many economically important species such as natural pacific salmon. Polycyclic aromatic hydrocarbons (?PAH_{diss}) at surface water of Okhotsk Sea were firstly studied in 1993 by Nemirovskaya. ?PAH_{diss} ranged from 2 to 8 ng L⁻¹, except for the northeast of Sakhalin where offshore oil extraction takes place (?PAH_{diss} reached 338 ng L⁻¹). The latest report (Chizova et al., 2013) from Russian side was from 2006, northern of the Sakhalin island, where ?PAH_{diss} were 33-78 ng L⁻¹. Research on the environmental behavior of organic pollutants is essential for assessing ecological risks of anthropogenic stressors to marginal seas, especially when transboundary transport may be implicated. The Southern Okhotsk Sea is fed by three main oceanographical currents; Forerunner Soya Warm Current (Spring) and Soya Warm Current (Summer) starts at Soya Strait while East Sakhalin Current (Autumn-early Winter) starts northern of the Sakhalin Island. The present study aimed to evaluate the impact of PAHs surface migration around Southern Okhotsk Sea by the monitoring of the surface distribution of PAHs in seawater. More than 20 samples (~10 L) were collected from July 2017 to September 2021 with collaboration of the Japan Fisheries Research and Education Agency. Particulate and dissolved phases were separated by 0.5 µm pre-combusted glass-fiber filters and dissolved PAHs were concentrated using C18 disk. PAHs were measured in a HPLC-fluorescence system. The ?PAH_{diss} averaged 1.87 ng L⁻¹ (1.7-4.4 ng L⁻¹). Using isomers ratios, sources were inferred to be majorly pyrogenic in all samples. ?PAH_{diss} vs Salinity plot suggest that dissolved PAHs distribute into a high salinity (> 32.4 PSU) / low PAHs (< 3 ng L⁻¹) coastal

area and a low salinity / high PAHs offshore area. However western of Soya Strait, Σ PAH_{diss} were not necessarily low (2.85-5.56 ng L⁻¹) between 2017 and 2019 at summer seasons. Coastal and offshore areas are also characterized by their nutrients loads and primary productivity. The former has higher chlorophyll-a (1.2±1.2 mg m³) and less inorganic nutrients (PO₄ 0.13 ± 2.8 μM, SiO₂ 3.5±2.8 μM) while the latter has lower chlorophyll-a (0.6±0.5 mg m³) but more inorganic nutrients (PO₄ 0.29±0.11 μM, SiO₂ 13.0±3.9 μM). Preliminary, we conclude that surface migration does not fully explain PAHs distribution at Southern Okhotsk Sea, and factors such as bio-/photo-degradation and scavenging could be equally or more important.

6.08.P-Mo272 The Derivation of a Maximum Acceptable Concentration (MAC) for Azamethiphos

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Azamethiphos (CAS No. 35575-96-3) is an organophosphorus pesticide, which acts on insects and other arthropods by inhibition of acetyl cholinesterase activity. It has been authorised for use in Scotland as a veterinary medicine for the control of external parasites in farmed fish for over 20 years. Regulatory Maximum Acceptable Concentrations (MAC) for application to the marine environment in the vicinity of Scottish fish farms are available for azamethiphos (SEPA 1998). These Environmental Quality Standards (EQS) incorporate a temporal element to account for the dispersal and dilution of azamethiphos over time in the local marine environment following its use to treat farmed fish, and represent the concentration of azamethiphos that should not be exceeded in the vicinity of fish farms at 3, 24 and 72 hours post-treatment. The short-term ecotoxicity dataset for azamethiphos has been updated to include all data that could be identified, obtained and confirmed as reliable and relevant for EQS derivation. This dataset now covers a large number of species and higher taxonomic groups. This updated short-term ecotoxicity dataset has been used to derive new MAC values for azamethiphos according to the current European guidance on EQS derivation (EC 2018). Since generic MACs derived in this way do not incorporate a specific temporal element such as are required to assess environmental risk at defined time periods post-treatment of fish farms with azamethiphos, updated fish farming-specific MAC have also been derived, using the approaches previously applied for the derivation of such temporally-defined MACs (SEPA 1998). This poster presentation will detail the dataset and assessments undertaken in deriving the MACs, and updated temporally-defined marine MACs will be proposed.

6.08 Tackling Marine Pollution on the Road to Sustainable Use of Marine Ecosystem (Poster Corner)

6.08.PC-Mo13 Chemical Warfare Agents (CWA) in the Baltic Sea Food-Web: Clark I Bioaccumulation Modelling Using ECOPATH With ECOSIM (EwE)

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Sea dumping of chemical warfare in the Baltic Sea area took place soon after the World War II and resulted in disposal of approx. 50 000 tons of CW containing up to 15 000 tons of chemical warfare agents (CWA). There is a growing concern regarding the environmental impact of weaponized compounds that are now known to be environmentally persistent and very toxic for model aquatic organism *D. magna*. Furthermore, one of the recent discoveries puts phenylarsenicals in the spotlight due to the first detection of CWAs degradation products in fish samples collected in the CW Primary Dumpsite in the Bornholm Deep area of the Baltic Sea. The aim of this study was to simulate possible pathways, behaviour and future spreading of Clark I in the Baltic Sea food web under various scenarios. This has been achieved by putting together all existing information about the CW dumping loads and laboratory-derived measurements with *in-situ* observations and measured values of detected bioconcentration of Clark I to perform bioaccumulation modelling using ECOPATH with ECOSIM Baltic Sea Food-Web Model with Ecotracer module. Scenarios simulations are based on chain of biogeochemical and ocean-climate models for the Central Baltic Sea. Separate modelling scenarios were performed for two reported loads: 711 and 1500 tons of Clark I. When normalized by the real-data organic matter content in the sediments from contaminated areas of the Baltic Sea, the modelled accumulation of Clark I by detritus never exceeded the highest detection in analyzed sediments. Regardless of the applied release ratio for Clark I it reaches the concentrations per biomass matching real measurements in adult cod already in first months of modelling and increases over time reaching top predators. As assumed, various socio-economic scenarios, including the type and extents of fishing result in different rates of contamination spreading and influence vectors and residues of contamination in various functional groups. *Saduria entomon*, Atlantic cod, Grey Seal (*Halichoerus grypus*) are the contamination hot-spots, while due to the detritus-based pathways, several pelagic species and fish-feeding birds seem not to be significantly affected by Clark I bioaccumulation. Studies provide a novel and interesting insight into future contamination spreading and indicate new target species for future monitoring of this particular type of xenobiotics in the Baltic Sea food web.

6.08.PC-Mo14 An Interdisciplinary Approach to Assess the Impacts of Shipping Emission Control Technologies on the Marine Environment: EMERGE Project and the Northern Adriatic Case Study

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Following the adoption of the global limits on sulphur content for marine fuels, the use of shipping emission abatement technologies such as exhaust gas cleaning system (scrubbers) has been predicted to increase significantly in the next decades as an alternative to the use of cleaner but more expensive fuel types. Since acidified and contaminated scrubber discharge water are presumed to cause a deterioration of the marine environment, there is the need for an integrated assessment framework to evaluate the impacts of these emissions on marine ecosystems, while considering also the environmental and health benefits of reducing emissions of air pollutants, and associated costs. This is the main objective of the H2020 EMERGE project, which evaluates

environmental and health impacts of shipping emissions reduction technologies under future scenarios by integrating modelling and experimental approaches. The EMERGE framework is being applied to five EU case-studies, including the Northern Adriatic Sea, a shallow marine basin that includes zones of high ecological value and hosts various anthropic activities such as fishing, aquaculture and port activities, which, along with land-based activities, pose chemical pressures on the marine environment. The first component of the modelling chain is the STEAM model, which provides shipping emission estimates of pollutants and nutrients based on Automatic Identification System data and includes updated emission factors from scrubber water analysis. High resolution models are used for predicting the transport of selected substances in the air and water compartments, and the obtained fluxes of contaminants in the case study area are then used for investigating: (i) the effects of changing nutrients loads on biogeochemical processes using the Biogeochemical Flux Model, (ii) the concentration, persistence, and fate of target pollutants using the OpenDrift suite and (iii) the bioaccumulation of pollutants using the MERLIN-Expo model. Ecotoxicological tests carried out on real scrubber water using planktonic bioindicators (i.e. *Aliivibrio fischeri*, *Acartia tonsa* and *Mytilus galloprovincialis*) and considering different life stages and exposure times will complement the modelling activities and support the comprehensive impact assessment for the case study area.

6.08.PC-Mo15 Development of Indicators Related to Chemical Contamination of Marine Biota (Fish, Birds, Mammals) Within the Marine Strategy Framework Directive

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Good Environmental Status (GES) for the descriptor 8 (D8) of the Marine Strategy Framework Directive is reached when concentrations of contaminants are at levels not giving rise to pollution effects. Offshore chemical impregnation by both inorganic (metals) and organic contaminants (e.g., polychlorinated biphenyls) is monitored in three group of species: fishes, birds and mammals. An indicator is defined by the couple of matrix*substance at the subregional level, the objectives of this study are: 1) to develop indicators for each group of species in offshore waters (as defined in the present study, as the waters beyond 1 milles or the water framework directive (WFD) waterbodies); 2) to develop an integrated method to assess GES achievement for criteria 1 of D8 in biota in offshore waters including the three developed indicators. Each indicator is built in three steps: (i) a contamination index for each studied species which is the upper limit of 95% confidence interval of the mean value based on substance concentration measured in fishes muscle or birds feathers. For mammals, metals concentrations are measured in liver or kidney, and in blubber for polychlorinated biphenyls; (ii) a contamination ratio, informing on the distance to thresholds, is calculated as the ratio between the contamination index and the thresholds. Thresholds used to assess whether concentration could harm marine life, are the ones developed within regional sea convention and the Environmental Quality Standard (EQS) for secondary poisoning for Hg in fish. (iii) a contamination score, integrating the different species in one group of species, is determined by the sum of contamination ratio of each species divided by the square root of the number of contamination ratio. To achieve GES for each matrix, contamination score has to be lower than 1. To assess GES at biota level in offshore waters, the one-out-all-out method is used. Preliminary results in Mediterranean French seas showed that concentrations in mercury are higher than thresholds in fishes and in birds with a contamination score equaled to 132.2 and 1.03, respectively. Therefore, for mercury, GES at biota level in offshore waters is not reached.

6.08.PC-Mo16 New Biological Effect Indicators in the Nationwide Monitoring Programme for the Aquatic and Terrestrial Environment (NOVANA) in Denmark

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The strategy for monitoring of biological effects (BE) of contaminants in biota in marine areas employed in NOVANA, and promoted in international conventions as HELCOM (Baltic Marine Environment Protection Commission) and OSPAR (Oslo and Paris Convention), is integrated environmental monitoring. Within the frames of this monitoring, chemical measurements in marine biota and sediment are conducted in parallel with BEs, in selected species of organisms. Different types of BE indicators are included in the marine part of the NOVANA in Denmark (1) and the Danish Marine Strategy (2), as part of Descriptor 8. Here we present the results in development of a set of biological effect indicators that have potential to be included in NOVANA - biliary metabolites of polycyclic aromatic hydrocarbons (PAHs) in fish species, i.e. european flounder (*Platichthys flesus*), round goby (*Neogobius melanostomus*), and malformed embryos of amphipods (*Gammarus spp.*). PAHs are widespread contaminants in the marine environment, and PAH metabolites in fish bile and enzymatic CYP1A activity in fish liver (EROD) are established and internationally identified as relevant indicators for e.g. HELCOM and OSPAR assessments. The results presented, include response levels in PAH metabolites and EROD in european flounder and round goby, as alternative to viviparous eelpout (*Zoarces viviparus*), which is currently employed within NOVANA, since the use of eelpout is challenged by locally decreasing populations. SFS (synchronous fluorescence spectrometry) and HPLC/F (high-performance liquid chromatography with fluorescence detection), were used for identification and quantification of three specific types of PAH-metabolites – 1-hydroxy-compounds of phenanthrene, pyrene and benzo(a)pyrene. Amphipod embryos are sensitive to pollution exposure during the embryogenesis (3), and therefore the reproduction in amphipods as BE indicator is recommended within HELCOM. We present results on Danish pilot study where embryo aberrations were examined according to ICES TIMES guidelines (3). 1. 1. Miljøstyrelsen, DCE, & GEUS. (2017). NOVANA - Det nationale overvågningsprogram for vandmiljø og natur 2017-2021. Programbeskrivelse. 2. 2. Miljøstyrelsen. (2020). Danmarks Havstrategi II Anden del Overvågningsprogram. 3. 3. Sundelin, B.,

Track 7: Think-outside-the-box (fundamentally new concepts, innovative and controversial ideas, and interdisciplinary issues)

7.01 Being prepared for the effects of future technologies – studying the environmental risk and life cycle impacts as well as the recycling potential of so-called Critical Raw Materials

7.01.T-01 REE Interactions With Organo-Mineral Colloids As a Control of the REE Environmental Dissemination

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Production of high technology products consumes large quantities of rare earth elements (REE), and subsequently increasing their extraction and dissemination. As a consequence, concerns have arisen about the environmental safety of using REE. The dissemination of rare earths in the environment is the result of transfer mechanisms that depend on environmental physio-parameters and REE speciation, which is largely controlled by their binding to solid surfaces and notably to colloids. Here, we are interested in colloids of organic matter and iron (Fe) because they are ubiquitous and their abundance is increasing in natural waters in response to the global climate change. Recent studies have shown that their structural organization consists of Fe nano-beads with a fractal organization that leads to different aggregates associated with OM molecules or colloid. This organization, which evolves with the major cations, controls the reactivity of the colloids. Our objective is therefore to explore the intimate bonds between REE and these colloids in order to study their impact on REE mobility. Three different colloids were synthesized using Leonardite humic acid (OM) and Fe(II) at a Fe/ OM molar ratio of 0.25 without or with calcium (Ca) used as a coagulant (Ca/Fe ratio 0.1 and 0.5). Adsorption experiments of REE by colloids (REE/Fe ratio from 0.1, to 0.0008) were carried out at pH 6.5. The adsorption of REE varied between 50 and 99% with the decreasing REE/Fe ratio. The adsorption patterns exhibited a middle REE downward concavity, indicating that REE were potentially bound to both the Fe phases and OM. At low REE loading, the pattern on the colloids is controlled by small but strongly complexing organic molecules (< 3kDa). Calcium weakly promotes REE adsorption by colloids by increasing the availability of the colloid binding sites, which is due to a decrease in the interactions between the Fe phase and OM. Finally, A4F-UV analysis showed that the addition of REE to the colloids affects their structural organization by promoting the formation of a colloids network. Although it must be admitted that colloids increase the mobility of the associated metals. The present study provides evidence that REE binding to Fe-OM colloids promotes the formation of a colloid network that severely restricts their mobility in the environment.

7.01.T-02 Sensitivity of Different Microalgae Species Towards Rare Earth Elements

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Rare earth elements (REE) are a series of chemical elements represented by 15 lanthanides, yttrium, and scandium. Their application in green and sustainable technology as well as their anthropogenic activities mean an increase in the global demand and concern about the environmental safety of these metals. REE are now labelled as 'emergent' contaminants due to their apparent disruption of biogeochemical cycles. Only a few studies who have looked into the toxicity of REE on microalgae. However, there it appears that a lot of different algae species are used for the toxicity assessment. Hence, the objective of the study presented here is the comparison of the sensitivity of 3 microalgae species with regard to lanthanum (La) and gadolinium (Gd). We hypothesize 1) similar sensitivity towards the two metals as the mode of action of REE is assumed to be similar. 2) a strong difference in sensitivity among species with *Scenedesmus vacuolatus* having the lowest sensitivity as it will be least affected by phosphate limitation as a consequence of REE-PO₄-complex formation. Toxicities to Gd and La were determined applying the algae growth inhibition (AGI) test in a DIN media with phosphate. Three different freshwater microalgae species, *Scenedesmus vacuolatus*, *Chlorella vulgaris*, and *Raphidocelis subcapitata* were exposed for 72 hours at nominal concentrations of 100, 10, 1, 0.1, and 0.01 mg/L of Gd and La. Biomass increase and growth rate were determined by fluorescence. *R. subcapitata* and *C. vulgaris* are species commonly used in ecotoxicology laboratories. *S. vacuolatus* was chosen because it is known for its ability to accumulate phosphate and store it internally. This makes it an interesting species for the algae growth inhibition test as Ln are known to form complexes with phosphates which may cause PO₄-depletion. Results indicate that Gd exposure causes higher absolute toxic values of up to 95% inhibition, while La leads to no more than 50% of fluorescence inhibition. The sensitivity of the microalgae species in this bioassay varies strongly (*C. vulgaris*, *S. vacuolatus* >> *R. subcapitata*.) Our hypothesis, that *S. vacuolatus* would be the least sensitive cannot be validated. Therewith, phosphate limitation does not seem to be the major cause of growth inhibition. We suggest that these observations are caused by differences in toxicokinetics or -dynamics of *R. subcapitata* compared to the other species used. We will elaborate further on this in the presentation.

7.01.T-03 Implications of Speciation on Rare Earth Element Toxicity Assessment: A FOCUS on Organic Matter Influence in Daphnia magna Normalized Test

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Rare Earth Elements (REE) became essential in high- and green-technologies. Their increasing use lead to the release of anthropogenic REE into the environment including aquatic systems. Limited data concerning REE aquatic ecotoxicology indicate their biological effects are highly dependent on their speciation, which prevents the establishment of a reliable environmental risk assessment. The current study assessed the influence of speciation on the toxicity of neodymium (Nd), gadolinium (Gd) and ytterbium (Yb) by the *Daphnia magna* mobility inhibition test (ISO 6341, 2012). Toxicity was assessed for each element individually and in ternary mixture, as well as in the absence and presence of dissolved organic matter (DOM). Speciation was predicted by modeling. REE bioaccumulation by *D. magna* was measured to better understand the relationship between REE speciation and toxicity. DOM decreased significantly the toxicity of Nd, Gd and the mixture towards the freshwater crustacean. This was explained by a lower REE bioaccumulation in the presence of DOM due to REE-DOM complexation, which reduced REE bioavailability. DOM effects on Yb toxicity and bioaccumulation were limited because of Yb precipitation and the potential contribution of Yb-hydroxide nanoparticles to Yb bioaccumulation and toxicity. We demonstrated that the way of expressing EC50 values changed drastically REE toxicity assessment, and that these changes were influenced by REE speciation. This study demonstrates for the first time that REE speciation, and especially REE-DOM complexation, influences significantly REE bioaccumulation toxicity towards *Daphnia magna*.

7.01.T-05 Is Lithium Toxic to Freshwater Organisms? Focus on the Zebra Mussel As a Representative of Filter-Feeders *Nicolas Dupuy¹, Laetitia Minguez² and Carole Cossu-Leguille³, (1)LIEC - Université de Lorraine - CNRS, Metz, France, (2)LIEC (CNRS UMR 7360, Université de Lorraine), France, (3)Université de Lorraine, France*

Lithium is the first of the alkali metals. Due to specific physicochemical properties, this metal is increasingly used in a context of energy transition, particularly in the manufacturing of lithium-ion batteries. The demand is estimated to grow by more than 30% per year up to 2030, and the long-term sustainability of this resource is at risk. Lithium is thus considered as a critical material. Anthropogenic inputs of lithium in aquatic ecosystems have already been observed, and are likely to increase in the coming years. This increase raises the question of the potential impacts of lithium on chronically exposed organisms. Information about lithium biological pathways are more abundant for humans than for aquatic organisms, due to its use in medicine to treat bipolar disorders. Although several biological targets are documented in the literature, lithium mode of action shows great complexity, affecting several cross-related signaling pathways and complex cascades of events. Moreover, most of the data in the literature are acute toxicity data, and few of them are conducted with freshwater organisms. In this context, we studied the long-term effects of lithium in a freshwater bivalve, *Dreissena polymorpha*. Mussels were exposed for four weeks to four concentrations of lithium: 0, 40, 100 and 250 $\mu\text{gLi}^+/\text{L}$ as LiOH. Several physiological parameters were regularly assessed in parallel to exposure concentrations and bioaccumulation, related to energetic metabolism, antioxidant defenses and cell damages. Effects at the individual scale were assessed by monitoring the filtration rate, oxygen consumption and the scope for growth. We observed high mortality during the experiment, which was lower in lithium-exposed conditions than in controls. First results show a lower energy expenditure in organisms exposed to lithium than in controls. Analyses still in progress will allow us to depict an integrative vision of lithium interaction with exposed organisms, and to understand whether lithium is toxic to aquatic organisms or whether, in particular conditions, it can help them to cope with other stressors.

tbd

7.01 Being prepared for the effects of future technologies - studying the environmental risk and life cycle impacts as well as the recycling potential of so-called Critical Raw Materials (Virtual Only)

7.01.V-01 A Framework for Assessing the Damages of Resource Services Loss in LIFE Cycle Assessment

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Purpose Several modelling perspectives are identified in the literature to assess the impact of resources depletion in LCA (Sonderegger et al. 2019). Some methods consider the loss of an inherent property of resources, such as energy or exergy. Others assess additional societal efforts required in the future due to current extraction or the decreasing natural availability due to extraction. Today there is a consensus about the need to evaluate the instrumental value of resources to humans as described by the area of protection “Natural resources” in the life cycle impact assessment framework (Verones et al. 2017). Unlike proposed by some LCIA methods, extraction alone does not contribute to resource depletion. In fact, abiotic resources may remain in the technosphere and stay available for future uses. Therefore, all mechanisms affecting the loss of services should be considered and not restricted to the extraction from the environment, but also substitutability for the same function needs to be accounted for. This presentation aims to present a novel framework for assessing the damages related to the loss of instrumental value of resources in life cycle assessment. **Methods and Results** First, a cause-effect chain for the assessment of the potential loss of services provided by abiotic resources is proposed. It links the fate of resources to a potential service loss and then to its effect of potential recovery cost to be paid by society. Second, we present a systematic approach for the identification of services provided by each resource. It requires to (i) identify resources end-uses and (ii) map each end-use with a final service that answer to human needs. Finally, we propose an interface to link the proposed life cycle impact assessment (LCIA) framework with the life cycle inventory (LCI). It implies adapting the current structure of LCI databases by including new elementary flows that not solely cover extraction from the environment but also change in functionality and other losses within the technosphere. Then, both new elementary flows and the required additional information in EF, other than the name of the resource, that would enable the quantification of services gain or loss, is presented. **Conclusions** This framework can be useful to enhance a sustainable management of the provision of resource services that are contributing to human well-being.

7.01.V-02 Novel Empirically-Derived Freshwater Ecotoxicity Characterization Factors for Rare Earth Elements: Nd, Gd & Yb

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Rare Earth Elements (REE), used in industrial and human activities, find their way to aquatic ecosystems, where they can be potentially harmful for its species. In this study, we assess this cause-effect chain for Neodymium (Nd), Gadolinium (Gd) and Ytterbium (Yb). In life cycle assessment (LCA) methodology, the freshwater ecotoxicity impact due to a pollutant is equal to the released emissions amount multiplied by so-called Characterization Factors (CFs). The latter are provided by impact assessment methods, and USEtox is the consensual one to characterize ecotoxicity. While USEtox covers more than 3000 substances, CFs for Nd, Gd, and Yb are still lacking. This work aims at filling this gap for the cationic state of the REE, based on empirical data to represent more accurately CF for specific conditions. Material & Methods: In USEtox, the ecotoxicity CF is retrieved by multiplying a fate factor with a toxicity effect factor. For the first, partitioning coefficients are needed to specify the distribution among environmental compartments. Hereto, site-specific compartmental concentrations from measurements were used. The studied system is the upper Alzette River basin (LU) and the average between upstream and downstream measurements are considered retrieved in 2019, while for soil particles this was retrieved for 2007 through alluvial soil samples. The Bioaccumulation Factors (BAF) were retrieved through experiments for the fish *Danio rerio*, the crustacean *Daphnia magna* and the Bivalve *Corbicula fluminea*. For the ecotoxicity characterization, acute EC50-values for the three REE were experimentally derived using standard procedures for the following two species: the crustacean *Daphnia magna* and the alga *Raphidocelis subcapitata*. The use of chronic-to-acute ratios led to estimates for chronic ecotoxicity. Results & Discussion: The obtained CFs are consistent with USEtox CFs for metals. The differences between the three REE is mostly influenced by fate factors (higher affinity for soil, sediment and DOC leads to lower exposure to freshwater species) and could be explained by their typology (light, medium or heavy REE). Further reflections are needed to fully understand the results, in particular due to the complexity of REE chemistry in ecotoxicological test media (e.g. bonding with (in)organics). We will further perform uncertainty & sensitivity analyses to better specify influential factors, and also better compare with the results of other metals.

7.01 Being prepared for the effects of future technologies – studying the environmental risk and life cycle impacts as well as the recycling potential of so-called Critical Raw Materials (Poster)

7.01.P-Mo273 A Proof-Of-Concept Study on Potential Per- and Polyfluoroalkyl Substance Emissions From Relevant Recycling Processes of Lithium-Ion Batteries

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In 2019, the Nobel Prize for Chemistry was awarded to the development of the lithium-ion battery (LIB) – an invention that is considered to be a main element in the increasing electrification of society and a key component for overcoming the ongoing climate crisis. But even this promising application, which can facilitate the transition to fossil-free transportation, is facing environmental and ethical challenges, in particular with regards to metals scarcity. Recycling of LIBs in order to recover and re-use precious materials plays an important role in moving towards a circular society. However, the recycling process is still under development, and the by-products from this process (some of which may be hazardous) are not fully characterized. The use of fluorinated components in LIBs represents a particular concern. The so-called per- and polyfluoroalkyl substances (PFAS) are a broad class of synthetic chemicals which are highly persistent and mobile. Some well-studied PFAS have been detected in wildlife and humans globally and some have been linked with adverse health effects. Consequently, several PFAS have been regulated internationally in order to prevent further emissions. This study aims to investigate potential formation and emissions of PFAS during LIB recycling using a fluorine mass balance approach. Samples were collected at different steps in the recycling process of common LIBs (e.g. LiNiMnCoO₂), including the recovered electrolyte components, exhaust gas cleaning solutions, and shredded materials from the pre-treatment process (black mass). In order to capture the broadest range of organic and inorganic fluorine species, samples were extracted sequentially with solvents of different polarities, and then characterized for organofluorine (EOF), target PFAS, and suspect PFAS. Total fluorine was also determined in unextracted samples. This study provides clarity into recycling processes of fluorinated materials, thus indicating if post-treatments or change of conditions are necessary to avoid the formation and emission of PFASs. Additionally, the results can indicate if LIB research should focus on fluorine-free materials to help phase-out PFAS, and guarantee more environmentally friendly applications.

7.01.P-Mo274 Probing the Effect of Aluminium Substitution in Goethite on Rare Earth Element Adsorption

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The extraction and use of Rare Earth Elements (REE) has been increasing at a rapid pace in the last couple decades due to their critical importance in the technological sector. As a result they have been deemed an emerging pollutant. However, little is known about REE's behaviour in the environment. There have been studies done on REE adsorption on Fe (hydr)oxides, but they were performed under lab conditions using pure phases. Since REE adsorption and patterns vary according to the adsorbent phase, one

can expect that substitutions in a given structure would impact its adsorption capacity. As aluminium (Al) is a major element of the Earth surface that can easily enter Fe-oxihydroxide structure, this study looks at the effect of Al substitution in goethite to see if and how it may affect the REE adsorption; goethite being chosen as a well crystallised phase allowing modelling of sorption and surface interactions. For this purpose, a pure goethite and a goethite with 5% Al substitution were synthesized. The impact of Al substitution on their structural organisation was characterized by TEM, XRD, and XAS. The results show that Al enters into the goethite structure, forming clusters, without modifying the goethite needle shape but slightly increasing the specific surface area and the point of zero charge. Hereafter, REE adsorption kinetics and isotherms were performed to determine the effect of Al substitution on REE adsorption. This was done by measuring the REE in solution with an ICP-MS. The results show that even an Al substitution rate as low as 5% has an effect on REE adsorption. Substitution causes an overall decrease in adsorption of REE, although both adsorbents reach the adsorption equilibrium after 24h. In addition, while the MREE and HREE are still enriched compared to the LREE, the enrichment is less pronounced. The impact of Al substitution on the REE surface binding was investigated by XAS. These results evidence the impact of even low Al substitutions in Fe-oxihydroxides on their adsorption and thus on the fate of REE as pollutants in the environment.

7.01.P-Mo275 Influence of pH in the Toxicity of Rare Earth Elements Lu, Sm, Y, and Yb to Model Organisms

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Rare earth elements (REEs) have become vital in today's technology as they are widely used in the manufacture of ceramics, magnets, petroleum, electronics, among other uses. With the increase of their importance in today's technology, they also have become the focus of many studies, being recognized as emerging contaminants. However, because of the rapid increase of the environmental concentrations caused by the extraction processes and the electronic waste, there is still a gap in the knowledge related to their ecotoxicological effects. This study aims to contribute to filling the gaps of the associated individual REEs toxicities. Therefore, the focus of this study is the ecotoxicological effects of four of the least studied REEs: Lu, Sm, Y, and Yb on bioassay model organisms of different groups of the trophic level (e.g., *Daphnia magna*, *Raphidocelis subcapitata*, *Sinapis alba*, and *Alivibrio fischeri*). To evaluate the effects of REEs under laboratory conditions, the organisms were exposed to various ranges of concentrations diluted with the organism's culture medium to determine the relative concentration-response curves. Scenarios including acidic pH values (i.e., acid mine wastewater) were considered. Preliminary results indicated: 1) higher toxicity at lower pH values; 2) Sm as the element with the highest toxicity, followed by Y, Yb, and Lu; 3) *A. fischeri* as the most sensitive species, followed by *R. subcapitata*, *D. magna*, and *S. alba*, in decreasing order.

7.01.P-Mo276 Rare Earth Element Speciation and Bioaccumulation in Estuarine Ecosystem

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Rare earths elements (REE) are a group of 17 metallic elements used in various industrial and medical applications. For several years, their use has been expanding rapidly, leading to numerous observations of enrichment in aquatic ecosystems. This study focused on studying the concentrations and speciation of REEs in the abiotic compartment of the Loire estuary ecosystem: sediment and water column (dissolved and particulate phases) in order to qualify the REE bioavailability for organisms. Their bioaccumulation by two species of benthic fish, the European eel (*A. anguilla*) and the Common flounder (*P. flesus*), revealed intraspecific and interspecific variations in muscle concentrations. In addition, the quantification of REEs in different organs and tissues allowed to determine their organotropism in eels. A particularly marked bioaccumulation of gadolinium in the gonads of female silver eels was thus demonstrated. Finally, a spatiotemporal study conducted on eel muscles showed a slight influence of the fishing area, but a significant temporal increase of REE concentrations between 2011 and 2019. This temporal difference was not observed in sediments collected at the same periods. The status of eel as a sentinel species is thus underlined, since it seems to be particularly suitable for monitoring the evolution of REE contamination in dynamic environments, such as estuaries, influenced by current and tides.

7.01.P-Mo277 Ree-Enriched Sediments Impacts on Life History Traits of Gammarus fossarum (Crustacea Amphipoda)

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The growing importance and use of rare earth elements (REE) are attracting interest from society, policy makers and scientists. The intense mining activities for commercial purposes is globally increasing and leading to the disruption of REE biogeochemical cycles with anthropic abnormalities already observed and reported in aquatic ecosystems. Nonetheless, REE remain less studied and their mechanisms of toxicity actions are not fully understood. As amphipods, *Gammarus fossarum* represent an important part of the aquatic macroinvertebrate assemblages, present a large distribution, and are generally used in ecotoxicological studies for their high ecological relevance (i.e., leaf litter breakdown). However, their use for the study of REE effects has been rather limited so far. The aim of the current study is the assessment of potential effects of two naturally REE-enriched sediments on *G. fossarum*. Effects on life history traits, behavioral and physiological responses of *G. fossarum* have been evaluated. Two experiments were performed on a water/sediment system. REE-enriched sediments were selected based on the concentrations of their parent's material and were collected in North Quebec, Canada. First, *G. fossarum* males were exposed for 72h to two naturally-REE enriched sediments, namely N2 and B4, and one control group. Physiological (osmoregulation) and behavioural (locomotion and ventilation) responses were evaluated. The second experiment consisted on exposing *G. fossarum* pre-copula

pairs with females at the same reproductive stage to the naturally REE-enriched sediments, for one molt cycle duration (~30 days) to assess potential effects on life history traits such as survival, growth and reproduction. The short-term exposure of *G. fossarum* males to sediments N2 and B4 led to a decrease in haemolymph osmolality and number of gammarids in movement while an increase in ventilatory activity was observed. The chronic exposure led to a significant uptake of REE and a significant decrease in proportion of female with oocytes and total number of juveniles were observed in organisms exposed to sediment B4. The physico-chemical analyses of sediments showed that B4 contains the highest amount of REE with a higher proportion of light REE. The present study gives first insights about the potential toxicity of REE on *G. fossarum* as they may have deleterious effects on *G. fossarum* population's dynamics, which may alter the functioning of aquatic ecosystems.

7.01.P-Mo278 Lithium Ecotoxicity in Aquatic Environment: A Meta-Analysis to Identify Gaps

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Lithium is an alkali metal naturally found in brines and minerals of some rocks (pegmatites, granitic domes and greisens). This metal is historically used in the treatment of psychological disorders such as bipolarity. Because of its particular physicochemical properties (high energy density and lightness), lithium is also increasingly used in a context of energy transition, particularly in the manufacturing of lithium-ion batteries. A contamination of aquatic environments is likely to be expected in the coming years. What will be the impact of this contamination on aquatic organisms? To answer this question, a meta-analysis was conducted on the data available in the literature, in order to identify gaps of knowledge about the bioaccumulation and ecotoxicity of lithium in aquatic environments, and to better understand the mechanisms underlying these phenomena. We also completed the existing literature by performing standardized tests on the decomposer *Aliivibrio fischeri*, the primary producer *Raphidocelis subcapitata* and the primary consumer *Daphnia magna*, with different lithium salts used in industry. The available data on bioaccumulation and ecotoxicity of lithium in aquatic environment show a strong heterogeneity. Although most EC50 values are above average environmental concentrations, very few chronic toxicity data are available. The organisms we used for our standardized tests showed a range of sensitivity comparable to the ranges found in the literature, and our results highlight differences in toxicity between salts and tested organisms. Concerning lithium bioaccumulation data, some studies report an accumulation of lithium similar to other elements known to be toxic (As, Cd). However, information is scarce on lithium bioaccumulation in freshwater organisms, which are potentially more exposed to lithium discharges because they are closer to anthropogenic sources. This work highlights the need to assess the ecotoxicity of different forms of lithium and to better understand their mechanisms of action.

7.01.P-Mo279 Approaches to Reduce the Negative Influence of Aquafarming on the Biodiversity and Water Quality of Costal Ecosystems

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Our focus is to build up new complex aquatic systems, which can be stabilised under different environment conditions. The interaction of several species in one system gives us the chance to avoid monoculture systems with high risk of biological collapse. In a multi-complex system, resources can be shared between different species and wastewater can be strongly reduced. We are currently working on resource protection technology, where we bring aquatic, marine, and ecotoxicological sciences together. The basic idea behind this approach is that standardised systems that we know e.g., from the ecotox science allows us to calculate environmental risk and predict responses of animals and plants to environmental change. This knowledge is useful for assessing potential risks in aquaculture and simulating effects under laboratory conditions. It is now very apparent that climate change significantly affects most ecosystems resulting in potentially dramatic changes of food webs. Our focus is to effectively use aquaculture and at the same time protect natural resources. This might be realised by circulation systems with minimum input of natural resources and avoidance of waste products having toxic effects on the environment. Here we present first circulation systems combining algae, water plants, invertebrates, and fish species from salt and freshwater ecosystems.

7.01 Being prepared for the effects of future technologies – studying the environmental risk and life cycle impacts as well as the recycling potential of so-called Critical Raw Materials (Poster Corner)

7.01.PC-Mo19 Evaluation of Chronic Toxicity of REE on Life Cycle and Proteomic Responses of *Daphnia magna*

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Rare earth elements (REE) are considered as emerging contaminants due to their large-scale exploitation and use in the high-technology sector. So far, no regulatory thresholds are available for REE. Compared to trace elements with well-documented environmental impacts, REE remain less studied and their toxicity mechanisms are not fully understood. Therefore, the aim of the current study is the investigation of chronic toxicity of REE on the reproductive cycle of the model organism *Daphnia magna* and the study of their molecular toxicity mechanisms through a proteomic approach. Effects of REE were investigated using a non-targeted approach at proteome level of *D. magna* juveniles (shotgun analysis). In this study, *D. magna* were exposed for 21 days to up to 600 µg.L⁻¹ to a mixture of Gadolinium (Gd), Neodymium (Nd) and Ytterbium (Yb). Effects on survival, growth rate, bioaccumulation and reproduction have been assessed. At the end of the exposure, juveniles from the fifth brood were kept and exposed for 48h to up to 600 µg.L⁻¹ to REE mixture (Gd, Nd and Yb). Effects of REE on protein expression have been assessed. Long term exposure of *D. magna* to the mixture of REE led to a decrease of the number of juveniles produced by organisms exposed to 600 µg.L⁻¹ of REE. Proteomic analysis allowed the identification of 862 proteins among which 171 were differentially

expressed among exposure conditions. REE led to the alteration of expression of protein modifying enzymes and metabolite interconversion enzymes. The highest concentration of REE (600 $\mu\text{g}\cdot\text{L}^{-1}$) led to the alteration of expression of calcium-binding proteins. This study gives first insights about the chronic toxicity of REE and their potential impact on *D. magna* population dynamics. Further analyses are ongoing at the gene expression level to identify the molecular mechanisms of action of REE on *D. magna*.

7.01.PC-Mo20 Using Biomass As an Alkaline Agent in Remediating Acid Mine Drainage From the Iberian Pyrite Belt and Removal Efficiencies for Rare Earth Elements

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The continuous generation of acid mine drainage (AMD) has a severe impact on the environment because of having low pH (<~3) and high concentrations of Fe, Al, and sulfates. In addition, Rare Earth Elements (REE), which are considered as technology critical elements, are also present in higher concentrations than those found in natural water bodies like for examples, rivers, lakes and oceans. All these factors together make AMD highly toxic and thus remediation is deemed a necessity. Remediation of AMD generated particularly from abandoned mines is typically achieved through the establishment of passive treatment systems (PTS) in which alkaline reagents are often used to neutralize the pH and to precipitate metals. Replacing commercial alkaline reagents by industrial waste products is an economically and environmentally promising alternative considering the reduction of waste volumes and the cost associated to alkaline reagents. One promising industrial waste product is biomass ash (BA) whose efficiency in remediating AMD generated at the Iberian Pyrite Belt (IPB) while retaining REE were tested in this study and compared with a commercial $\text{Ca}(\text{OH})_2$ reagent. In doing so, AMD samples collected from Poderosa mine (PM) located in IPB were first kept in contact with BA at different solid:liquid ratios of 1:2, 1:5 and 1:10. At different time intervals, supernatants were separated from the solids by centrifugation and analyzed using ICP-OES and ICP-MS. Removal efficiencies estimated for Al, Fe, Zn, Cu and REE. In a different set of experiment, PM were neutralized by the continuous addition of a solution 0.01 M $\text{Ca}(\text{OH})_2$. BA showed high removal efficiencies for Al (=99.9%), Fe (=98.9%), Zn (=99.9%), Cu (=99.9%) and REE (=99.9%) at the solid:liquid ratio of 1:2 similar to efficiencies obtained using 0.01 M $\text{Ca}(\text{OH})_2$. At this solid:liquid ratio, BA was able to neutralize pH within 96 hrs until a value of 7. This indeed shown that the BA is suitable to be used as an alkaline agent in treating highly acidic and metal rich AMDs and in retaining REE. The high retention of REE indicates the possibility for recovering REE contained in the AMD. Further, precipitated solids are to be characterized by XRD, SEM and LA-ICP-MS to identify the nature of the precipitated solids and the association of REE with the mineral phases contained.

7.01.PC-Mo21 Assessing Supply Disruption Impacts of Future Technologies Within Life Cycle Sustainability Assessment

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Switzerland and 193 other states have declared to become carbon neutral by 2050 by signing the Paris Agreement. An important step towards reaching this goal is to implement and upscale clean future technologies such as photovoltaics or batteries. However, these technologies often have complex supply chains, which may be disrupted by events such as the COVID-19 pandemic, the Brexit and the China-US trade war causing demand surge or supply reduction along supply chains. Existing criticality assessment approaches for raw materials allow for evaluating potential supply disruption impacts, but mostly for the early supply chain stages and in the short-term (i.e. next 5 years) only. In the framework of a Swiss National research project, the SPOTTER approach has been developed - representing the integration of criticality as an additional impact assessment aspect into the Life Cycle Sustainability Assessment framework. SPOTTER allows assessing supply disruption impacts along the complete supply chain of product systems in the medium-term (i.e. in 5 to 15 years). Within this approach, future supply scenarios are modelled and country-specific inventory flows are defined for individual product systems, each representing one selected technology consumed in a specific country. The inventory flow amounts are then multiplied with respective regionalized characterization factors to determine impact scores from different supply disruptions for each unit process along the supply chain of these technologies. Unit processes represent here single country-specific supply chain processes. Finally, relative supply disruption impacts and hotspots along the supply chains are illustrated for the different technologies as well as uncertainties of the impact scores, which have been defined using a Pedigree matrix and Monte Carlo Analysis, are visualized. In the frame of this presentation, results from the application of the SPOTTER approach are presented showing supply disruption impacts and related uncertainties as single scores and in boxplots, respectively as well as supply disruption hotspots on global maps. The hotspots and impacts are assessed along supply chains for strategically relevant technologies consumed in Switzerland including amongst others electric vehicles and photovoltaics. Supply chains of amongst others Rare Earth Elements and cobalt as well as supply disruptions caused by for example demand growth, resource depletion and limited recycling content are analyzed.

7.02 Characterizing the chemical (eco)exposome and associated environmental and health risk throughout the life span in the era of omics

7.02.T-01 Bioanalytical Measurement of Passive Sampling Extracts of Different Organs From Marine Mammals

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In their natural habitat marine mammals are exposed to a large number and variety of environmental pollutants. To understand how these complex chemical mixtures of pollutants distribute and accumulate within the organism, different organs of marine mammals need to be examined. Studying the chemical burden of organs with key functionality like liver, kidney, brain and fat tissue can help in characterizing the internal exposure and effects of chemicals. Passive equilibrium sampling (PES) transfers environmental mixtures of chemicals into an extract without changing their composition, which allows one to study the mixture effects of environmental pollutants extracted from marine biota without major bias. Polymer samplers such as the silicone polydimethylsiloxane (PDMS) can be used for PES of nonpolar, hydrophobic organic chemicals in biota. Bioanalytical assessment of this extract then provides information about the activation of important toxicity pathways of the overall chemical mixture. In this study the mixture effects of PES-PDMS extracts from liver, kidney, brain and fat from five harbour porpoises (*Phocoena phocoena*), one common seal (*Phoca vitulina*) and one orca (*Orcinus orca*) were characterized with three *in vitro* cell-based reporter gene bioassays. To cover the cellular toxicity and metabolic pathways, effects on the activation of the xenobiotic metabolism (aryl hydrocarbon receptor (AhR) and peroxisome proliferator-activated receptor gamma (PPAR γ)) and the adaptive Nrf2-dependent oxidative stress response were evaluated. In all three bioassays and for all paired samples the observed mixture effects of the liver extracts (and partly kidney and brain extracts as well) were higher than those of the fat extracts. Activation of PPAR γ was elevated by a mean factor of 12.9 for liver extracts relative to the corresponding fat extracts. Activation of the oxidative stress response was also slightly reduced in fat extracts by a factor of 2.3. No activation of the AhR with the fat extracts were detected whereas all liver extracts triggered an effect. These observations indicate that the chemical activity in liver tissue is higher than in fat tissue. In this ongoing study we will further compare the results from the bioanalytical study to the chemical composition analyzed using gas chromatography/high-resolution mass spectrometry. Using iceberg modeling, we can then determine how much of the observed effect is explained by the detected chemicals.

7.02.T-02 In Vitro Bioassays for Detecting the Human Exposome

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Conventional biomonitoring programs have been criticized on the basis that they cannot include the full range of chemical pollutants that make up the exposome. Bioanalytical tools may therefore complement chemical analysis for cost-efficient and comprehensive biomonitoring in diverse tissue types. We investigated human post-mortem tissues (liver, brain, adipose tissue as well as blood) from 16 individuals after dynamic equilibrium sampling with polydimethylsiloxane by instrumental analysis with direct sample introduction (DSI) GC-MS/MS and bioanalytical profiling with reporter gene bioassays targeting the activation of the aryl hydrocarbon receptor (AhR) with the AhR-CALUX assay. The concentrations of persistent organic pollutants increased with age while non-persistent chemicals did not show any age dependence of concentrations in blood, liver and brain and were often present at lower concentrations in adipose tissue. The activity in the AhR-CALUX bioassay also increased with age and was higher in liver and brain than in blood. The sum of concentrations of the detected chemicals was positively correlated with the bioassay responses but mixture modeling showed that the detected chemicals explained less than 2% of the activation of the AhR and less than 0.5% of cytotoxicity. This means that more than 10,000 chemicals would need to be included in an analytical method to capture all the effects with many chemicals potentially being below detection limits but still contributing to mixture effects. This comparison demonstrates how *in vitro* bioassays are a powerful complement to chemical analysis that captures the complexity of the exposome.

7.02.T-03 Combined Effects of Chemical Mixtures Are Predictable for the Whole Transcriptome - a Proof of Concept Study With Zebrafish Embryos

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Humans and environmental organisms are constantly exposed to complex mixtures of chemicals. Extending our knowledge about the combined effects of chemicals is thus essential for assessing the potential consequences of these exposures. In this context, comprehensive molecular readouts as retrieved by omics techniques are advancing our understanding of the diversity of effects upon chemical exposure. This is especially true for effects induced by chemical concentrations that do not instantaneously lead to mortality, as is commonly the case for environmental exposures. However, omics profiles induced by chemical exposures have rarely been systematically considered in mixture contexts. In this study, we aimed to investigate the predictability of chemical mixture effects on the whole-transcriptome scale. We predicted and measured the toxicogenomic effects of a synthetic mixture on zebrafish embryos. The mixture contained the compounds diuron, diclofenac, and naproxen. To predict concentration- and time-resolved whole-transcriptome responses to the mixture exposure, we adopted the mixture concept of concentration addition. Predictions were based on the transcriptome profiles obtained for the individual mixture components in a previous study. Finally, concentration- and time-resolved mixture exposures and subsequent toxicogenomic measurements were performed and the results were compared with the predictions. This comparison of the predictions with the observations showed that the concept of concentration addition provided reasonable estimates for the effects induced by the mixture exposure on the whole transcriptome. Although nonadditive effects were observed only occasionally, combined, that is, multicomponent-driven, effects were found for mixture components with anticipated similar, as well as dissimilar, modes of action. Overall, this study demonstrates that using a

concentration- and time-resolved approach, the occurrence and size of combined effects of chemicals may be predicted at the whole-transcriptome scale. This allows improving effect assessment of mixture exposures on the molecular scale that might not only be of relevance in terms of risk assessment but also for pharmacological applications. The study is published in EHP: <https://doi.org/10.1289/EHP7773>.

7.02.T-04 A Step Forward Toward Harmonization of QA/QC Criteria for Chemical Exposomics - Lessons Learned From the HBM4EU Multi-Site SPECIMEn Study

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QA/QC provisions to assess the quality of data produced by suspect screening and non-target analysis workflows have been proposed and applied across five laboratories within the European Human Biomonitoring Initiative (HBM4EU) multi-site Survey on PEStiCide Mixtures in Europe (SPECIMEn) study. Within SPECIMEn, over 2000 urine samples were analyzed to investigate pesticide exposure in residents of five EU countries. The present work focused on the elaboration and application of harmonized QA/QC provisions to monitor the overall quality of the data generated and assess whether the raw data is acceptable for subsequent data pre- and post-processing. Standard solutions and fortified QC urines were prepared in a centralized way, distributed to all participants, and used to monitor the retention times, mass accuracy and signal intensities of selected QA/QC markers. Overall, the QA/QC procedures and quality of the data acquired provided an adequate basis for further data processing. Use of the proposed criteria allowed for the identification of analytical issues, instrumental failures, and sample handling errors. Space for improvement was identified, and refined QA/QC criteria proposed, building upon the lessons learned from the multi-laboratory study.

7.02.T-05 Endocrine Disrupting Chemicals in Plastic Food Packaging - In Vitro Toxicity and Chemical Composition

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While there is abundant scientific evidence that some chemicals used in plastic products, such as bisphenols, phthalates and nonylphenols, are endocrine disruptors, plastics are chemically much more complex. For instance, more than 10,000 substances are intentionally used in the production of plastics (e.g., plasticizers, antioxidants) and even more non-intentionally added substances (e.g., reaction byproducts, impurities) are associated with them. Since little is known about the chemical composition and the toxicity of these complex mixtures of plastic chemicals, this study aims at characterizing both using plastic food contact articles (FCA) as relevant sources of human exposure. Thirty-nine FCAs from Germany, Norway, South Korea the UK, and the US, covering the seven polymer types with the highest global market share were extracted and analyzed with reporter gene assays for a set of nuclear receptors (NRs) relevant for human health, including pregnane X receptor (PXR), peroxisome proliferator receptor gamma (PPAR γ), estrogen receptors alpha (ER α) and androgen receptor (AR). Further, these plastic extracts were analyzed using non-target high-resolution mass spectrometry to quantify the number of chemical features and tentatively identify the chemicals present in the FCAs. The chemicals extracted from 36 out of 39 FCAs activated or inhibited one or more receptors with activities up to 100% of the respective positive controls. The PXR was activated by 36, the ER α by 25, the PPAR γ by 23 samples, while the AR was inhibited by 14 of 39 samples. In total, we detected > 16,000 unique chemical features with the number of features per sample ranging from 37 to 9,936. Only 16% of the chemical features were tentatively identified using spectral libraries and *in silico* tools. Our results confirm that many FCAs contain endocrine disrupting chemicals that interfere with diverse NRs. Future research should aim at identifying the active compounds in these complex mixtures. Our work highlights the importance of analyzing whole mixtures of finished products and contributes to improving our understanding of plastics as a source of exposure to endocrine disrupting chemicals. *Acknowledgement* - This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 860720.

7.02 Characterizing the chemical (eco)exposome and associated environmental and health risk throughout the life span in the era of omics (Poster)

7.02.P-Tu307 Early-Life Exposure to Formaldehyde Through Textile Materials

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Clothes may contain a wide range of chemicals, most of them intentionally added. In recent years, there has been a growing interest in eco-friendly clothing, including the use of organic cotton. However, the process of eco-friendly fabric production may involve the use of toxic chemicals of special concern, such as formaldehyde, a known human carcinogen. The present investigation aims to determine the presence of formaldehyde and its associated dermal exposure risk in the eco-friendly and conventional clothing of pregnant women, babies and toddlers from the Catalan (Spain) market. In this study, 120 clothing samples bought in different on-line shops, stores and malls from Catalonia (Spain) were analysed. One-half of the samples corresponded to eco-friendly garments, while the remaining samples were conventional clothes. Garments from pregnant women,

babies aged < 1 year and toddlers aged < 3 years were considered within the investigation, as the human exposure study was focused on vulnerable population groups. Formaldehyde was detected in 30.6% of the samples, with a mean of 8.96 mg/kg. The two individual garments with the highest formaldehyde content were samples of pregnancy panties (55.7 and 37.3 mg/kg) followed by a sample of babies socks (24.5 mg/kg). Although the values found are within the legal EU limits (< 75 mg/kg), formaldehyde levels were surprisingly higher in the eco-friendly garments (10.4 vs. 8.23 mg/kg). However, these differences were only statistically significant ($p < 0.05$) for bras (11.6 vs. 7.46 mg/kg) and panties (27.1 vs. 6.38 mg/kg) of pregnant women. Dermal exposure and health risks were assessed for three vulnerable population groups: pregnant women, babies and toddlers. In general, the exposure was higher in toddlers (up to $1.66 \cdot 10^{-4}$ mg/kg/day). Both noncarcinogenic and carcinogenic risks were below safe limits, according to international regulations. Furthermore, although formaldehyde levels are below the legal limits (< 75 mg/kg) and health risks within acceptable ranges, clothing may contain other toxic substances apart from formaldehyde, thus increasing the risks. Therefore, future health risk assessments should be performed under multi-exposure and multi-chemical scenarios. This study is part of a large investigation focused on determining the co-occurrence of formaldehyde and other toxic substances (trace elements, aromatic amines, polychlorinated biphenyls and bisphenols) in clothing.

7.02.P-Tu308 Over 40 Years of Monitoring and Research for the Protection of a World-Heritage Estate From the Potential Impacts of Uranium Mining

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In 1979, the Ranger Uranium Mine began operating in the Alligator Rivers Region, which included the area now known as World-Heritage listed Kakadu National Park (Northern Territory, Australia). It followed a far-reaching parliamentary inquiry that recommended the establishment of a "Supervising Scientist" to oversee the environmental management of the mining operation, and provide scientific advice to the regulatory authorities and the stakeholders. Importantly, the stakeholders included the Traditional Owners of the land, the Mirrar First-Nation people, who wish to recommence their traditional hunting, gathering and ceremonial practices on the rehabilitated mine-site. Forty years on, the Supervising Scientist Branch has conducted a comprehensive research and monitoring program that has provided assurance to stakeholders that the health of the environment and people near the mine remains unimpacted. The operational phase of the mine officially ended when the last barrel of uranium oxide was packed in January 2021, and the rehabilitation of the mine is now the focus, which will require different research needs and monitoring approaches. This presentation will showcase the Supervising Scientist Branch's integrated research and monitoring program for receiving waters and demonstrate how this unique regulatory model has ensured that contemporary research practices are used for informed decision-making. It will cover the full life-cycle of the mine and show how risk-based frameworks facilitated the acquisition of the multitude of knowledge needed for the successful rehabilitation of the mine. The presentation will discuss how new technologies and leading-edge practices can potentially empower the Traditional Owners to conduct the monitoring of the mine following decommissioning. This presentation will demonstrate how the Australian government's investment in scientific expertise results in better environmental outcomes.

7.02.P-Tu309 Optimization and Monitoring of QuEChERS Simultaneous Multi-Residue Analysis method for Multi-Residue Analysis of Pharmaceuticals and Pesticides in Aquaculture Products

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Korea has the highest consumption rate of seafood in the world. Aquaculture represents the largest production in Korea's fishery production. However, pollution of the coastal area, where aquaculture farms are concentrated, threatens the safety of aquatic products. The aim of study was to optimize an QuEChERS analytical method for simultaneous determination of organic pollutants. The QuEChERS extracts were then measured using LC-HRMS. The target substances include 32 pesticides and 20 pharmaceuticals have not been regulated with maximum residual levels in aquaculture products. The method was validated according to CODEX guideline (CAC/GL 71-2009). Linearity of matrix-matched calibration curves were generated with $R^2 > 0.99$. Limits of detection (LOD) and limits of quantification (LOQ) for all matrices ranged from 0.1 to 2 $\mu\text{g/kg}$ and from 0.5 to 5 $\mu\text{g/kg}$, respectively. Intra-day ($n = 5$) and inter-day ($n = 9$) accuracy (i.e. recovery rate) and precision (RSD) were estimated at three different spiking concentrations (pharmaceutical: 5, 10, and 20 $\mu\text{g/kg}$; pesticide: 10, 20, and 50 $\mu\text{g/kg}$). All target compounds were satisfactory in terms of accuracy and precision according to CODEX guideline. The validated method was applied to aquaculture products ($n = 303$) collected from domestic farms. As a result, 14 pesticides and 8 pharmaceuticals were detected. The most frequently detected compound results revealed 3.7-35 $\mu\text{g/kg}$ of fluxapyroxad in saltwater fish, 3-46 $\mu\text{g/kg}$ of fluxapyroxad in freshwater fish, 1-2.5 $\mu\text{g/kg}$ of propafenone in shellfish, 2.4-60 $\mu\text{g/kg}$ of fluxapyroxad in crustacea, and 1.4-3.1 $\mu\text{g/kg}$ of terbutryn in seaweed. Further study should be followed to identify the source of the detected substances.

7.02.P-Tu310 Chemical Analysis of Micro and Nanoplastic in Human Blood

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Characterization of the chemical exposure often focuses on 'classical' organic contaminants and metals, among other classes of chemicals. In the past years, it has become evident that plastic particle fragments from synthetic polymer materials are prevalent in all environmental compartments. The widespread occurrence of micro and nanoplastics in our living environment fuels the expectation that human are exposed to these particles in their daily life. There is limited information on the effects of micro- and

nanoplastic particles (MNPs) on human health. Human exposure is evident by the detection of microplastics in drinking water, food, air, and evidence confirms the entry and presence of microplastics in the human system. The toxicity threshold of MNP ingested, in terms of physical characteristics such as shape, size, mass, and polymer composition is unclear. With increased usage of plastic products, the levels of microplastics in our living environment are expected to increase in the coming years. Thermo-analytical methods coupled with mass spectrometry allow for the simultaneous, selective, and sensitive analysis of micro(nano)plastic and permit quantification in addition to detection. Pyrolysis-gas chromatography coupled to mass spectrometry (Py-GC-MS) has been successfully applied for the simultaneous detection of several polymers in environmental samples. In this study we present an analytical method for MNPs in human blood which involve enzymatic digestion followed by microfiltration and analysis using Py-GC-MS. The method was validated through spiking experiments at concentration levels in the same range as the measured data. Pyrolysis parameters and GC separation conditions were optimised to improve the specificity and sensitivity of the method. Strict identification and quantitation criteria for the low detection limits of unique pyrolysates of polyvinyl chloride, poly(methyl methacrylate), polypropylene, polymer materials containing styrene units, polyethylene and polyethylene terephthalate were applied. Further aspects of calibration, recoveries, and potential matrix effects are discussed. Results from the pilot study showed that high production volume polymers applied in plastic were identified and quantified in blood.

7.02.P-Tu311 Analytical Workflow for Chemical Exposomics in Human Serum by Gas Chromatograph Mass Spectrometry

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The human exposome is defined as all environmental exposures throughout an individual's lifespan^[1], and techniques to measure it are of increasing importance with knowledge that most chronic disease has environmental etiology^[2]. The chemical exposome is a particular challenge because of 350,000 chemicals in commerce, and only a few hundred of these have historically been biomonitored in blood by targeted methods^[3]. To better understand health risks of the chemical exposome, a workflow was developed that combines sensitive multi-target quantification with nontarget discovery for environmental contaminants in small volumes of individual blood serum samples. An extraction and cleanup method was optimized for 106 target analytes (log K_{ow} range, 1.7 - 11.2) from 6 classes, including polychlorinated biphenyls, halogenated flame retardants, dioxins, polycyclic aromatic hydrocarbons, organochlorine pesticides, phthalate plasticizers. The absolute recoveries for the majority of targets with the optimized sample preparation method were in the range of 70-80%. Gas chromatograph (GC) - high resolution mass spectrometry (Orbitrap) parameters were tuned for maximum method sensitivity, and instrumental detection limits reach as low as 0.025 pg on-column. The developed workflow had high sensitivity, recovery, and reproducibility for target analytes and showed promise for simultaneous nontarget chemical discovery using an open-science data processing tool (MS-DIAL) and spectral matching to various public libraries (NIST, MassBank, GNPS). Application to strategic samples from Swedish cohort studies revealed inter- and intra-individual variation of the exposome, including based on lifestyle and dietary habits. References:[1] Wild, C.P.(2005) *Cancer Epidemiol. Biomarkers Prev.*, 14(8), 1847-1850.[2] Rappaport, S. M.(2010) *Science*, 330(6003), 460-461.[3] Wang, Z.(2020) *Environ. Sci. Technol.*, 54(5), 2575-2584.

7.02.P-Tu312 Emerging Contaminants in Shellfish and Sediments Along the Chinese Coastline

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Emerging contaminants (ECs) are chemicals that are not commonly monitored and not regulated in the environment, but many classes have known or suspected adverse effects on the environment and human health. There are many studies published in relation to their environmental occurrence and potential for toxic effects. However, the knowledge on the occurrence of ECs along the coastal areas of China is still limited. In addition, the coastal areas of China are key stopover sites for migratory shorebirds to feed and rest, many shorebird species are in serious decline potentially linked to habitat degradation along the Yellow Sea and Bohai Sea of China. As a part of the COAST-IMPACT project, the present study aims to comprehensively investigate the environmental occurrence of several ECs, including per- and polyfluoroalkyl substances (PFASs), parabens, organophosphate flame retardants (OPFRs), bisphenols and benzophenones (BPs), and benzotriazoles and benzothiazoles (BTs) in shellfish and sediments at key stopover sites along the coast of China. 67 shellfish and 37 sediment samples were collected in the intertidal mudflats of 13 sites along the coast of China (3 samples from each location) in 2018. The shellfish (soft-tissue) and sediments were freeze-dried and stored at -20 °C until analysis. Samples were pretreated with liquid-solid extraction and solid phase extraction and analyzed by LC-MS/MS analysis. The concentrations (in dry weight, d.w.) were in the ranges of < 0.12-2508 for PFASs, < 0.09-4179 for parabens, < 0.09-149 for OPFRs, < 0.036-279 for BPs and < 0.036-27.3 ng/g for BTs, respectively. The concentrations (in d.w.) in sediments were in the ranges of < 0.11-306 for parabens, < 0.015-64.4 for OPFRs, < 0.03-25.5 for BPs and < 0.03-99.2 ng/g for BTs (d.w.), respectively. The concentrations of parabens, OPFRs, and BPs in shellfish were higher than those in the corresponding sediments. Shellfish collected from Diaokou and Nanhaipu contained higher concentration of PFASs. Shellfish collected from Yalujiang, Panjin, Nanpu, Diaokou, Changyi west (all around the Bohai Sea) and Leizhou (South China) contained a higher concentration of BPs. The concentrations of parabens were higher in shellfish from Yalujiang, Panjin, Nanhaipu, Changyi west, Xiuzhenhe and Qidong (around the Bohai sea and Yellow sea). It was thus observed that the shellfish samples with high concentrations of ECs are mostly collected from the coast of the Bohai Sea. The geographic patterns of the occurrence of these contaminants were assessed and significant associations were established among the target analytes. ECs were found ubiquitous in shellfish and sediments along the coast of China, while concentrations in sediments were found lower than

those in shellfish (except for BTs). PFASs and parabens were the dominant ECs in shellfish. We will focus on the areas with high pollutant concentrations for further research.

7.02 Characterizing the chemical (eco)exposome and associated environmental and health risk throughout the life span in the era of omics (Poster Corner)

7.02.PC-Tu07 From Biotransformation Products to Altered Metabolites & Lipids - Lc-Tims-Hrms Combined With a Sophisticated Annotation Workflow for Xeno- and Endo-Metabolome Coverage of Zebrafish Exposed to Xenobiotics *Dimitrios E. Damalas¹, Eleni Panagopoulou¹, Adamantia Agalou², Dimitris Beis² and Nikolaos Thomaidis¹, (1)National and Kapodistrian University of Athens, Greece, (2)Biomedical Research Foundation Academy of Athens, Greece*

Given the large number of xenobiotics, there is an important gap in the literature concerning their adverse effects on aquatic organisms. The impact of xenobiotics in the aquatic ecosystem is evaluated in more depth when the whole xeno-metabolome (xenobiotics and their biotransformation products (bio-TPs)) and endo-metabolome (metabolites & lipids) of aquatic organisms is studied. HRMS-based workflows appear to be a powerful tool for the identification of bio-TPs and metabolites/lipids. Beyond the cases that identification fails because of reduced ionization efficiency, false negatives arise from the inadequate separation. Despite HRMS's high applicability and accuracy, separation of isomers/isobars is not always possible, as they may pose identical chromatographic and spectral profiles. Thus, identification remains a challenging task. Additional "dimensions" of separation are required to provide additional evidence and reliable identifications. Combining complementary chromatographic modes with HRMS seems a powerful alternative tool for the comprehensive and high-throughput identification of unknowns. Lately, Trapped Ion Mobility Spectrometry (TIMS) has been proved an up-and-coming technology, separating molecules based on their three-dimensional size and charge in the gas phase. The development of comprehensive annotation workflows that utilize the analytical evidence to their maximum is also imperative. The objective of this study is to highlight a high-end analytical platform (LC-TIMS-HRMS) that combines multiple dimensions of separation with HRMS, in order to provide extensive experimental evidence for the identification of bio-TPs and endogenous metabolites/lipids. In addition, we developed a data treatment workflow, consisted of suspect and non-target screening approaches combining different annotation tools. Overall, the aim was to highlight a holistic approach for comprehensive xeno- and endo-metabolome coverage to facilitate toxicity assessment of aquatic organisms exposed to xenobiotics. ZFE exposed to xenobiotics were used as case studies. The developed workflow encompasses a wide range of tools to identify i) bio-TPs of xenobiotics ii) endogenous metabolites and iii) lipids in the same dataset. Examples highlighting the added value of TIMS in the identification of bio-TPs will be presented. Finally, the annotation of several lipids will be showcased, leading to the observation of lipid accumulation as a toxic response to triclosan exposure

7.02.PC-Tu08 Enhanced Chemical Exposomics of Human Plasma by Phospholipid Removal and LC – HRMS

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The exposome is the sum of all environmental exposures over an individual's lifespan. Measuring the chemical exposome in blood is a challenging task due to its inherent chemical complexity and wide concentration ranges of endogenous and exogenous small molecules. Untargeted high-resolution metabolomics methods have been directly adapted for investigating the chemical exposome by liquid chromatography (LC) and high-resolution mass spectrometry (HRMS), thereby allowing detection of thousands of features. However, when it comes to human serum these signals are dominated by high concentrations of endogenous lipids. To achieve higher sensitivity for discovery and profiling of the chemical exposome, we hypothesized that enhanced chemical exposomics could be achieved with a sample preparation workflow focused on the removal of major phospholipid components. Here, following protein precipitation a simple solid phase extraction (SPE) step was optimized using small volumes of human serum. Satisfactory recoveries were maintained for multiple classes of environmental contaminants, including 70 of the major serum contaminants and their metabolites, such as flame retardants, per- and poly-fluoroalkyl substances, phthalates and pesticides. The resulting phospholipid-free extracts allowed injecting higher volumes of serum on column for LC-HRMS analysis, compared to routine HRMS methods, thereby assisting the detection of trace chemicals. Method limits of quantification (MLOQ) for the target analytes reached as low as 0.025 ng/ml for a 200 µL serum sample, making the method's sensitivity comparable or superior to targeted methods. Analytes were quantified or semi-quantified using isotope internal standards or the reference standardization method, respectively. An added benefit was the ability to profile endogenous steroid and thyroid hormones which may be impacted by the endocrine active component of the chemical exposome, thereby allowing measurements of exposure and effect in the same LC-HRMS analysis of individual biofluid samples. The selective nature of the phospholipid removal makes the method also suitable for suspect screening and unbiased untargeted analysis. Application of the method in cohort studies will be discussed with respect to variation of the exposome between and within individuals.

7.03 How to assess sustainability of chemicals and operationalize 'safe operating space for chemicals', 'do no harm' principle, and 'safe and sustainable by design' concepts?

7.03.T-01 Finding the Toxic Free Environment - Feasible?

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Finding a toxic free environment may seem like finding a needle in a haystack. For now. Or not. Who knows? The present research ventures into the environment, to find the needles out there. What can we learn from contemporary chemical pollution in surface water systems? What do the data tell us regarding concepts and themes such as the toxic-free-ness (or absence thereof), dominant chemical groups, spatiotemporal variability and alike, and on exceedance of thresholds of effects and the consequences of that? What do those lessons learn us on the needs for and approaches to safe and sustainable by design, and the zero-pollution ambition? Before carrying more haystacks full of uncertainties, the present research just looked at a snapshot ('photo') of chemical pollution, with Dutch surface waters as an example. We collected monitoring data from >8500 locations, to characterize current pollution. Mixture toxic pressures were quantified, and classified according to ecological impact phenomena. Results provided insights into the absence of toxic-free-ness, and spatial variability of dominant chemical groups. The toxic-free environment ambition would have been reached if we would have found no chemicals, no exceedances of local or regional thresholds, and no impacts. As we found variable distances to target, we asked ourselves not only about the causal agents, but also about systematic ways to explore the solution space. Solutions are 'inside the box', and we discuss how to identify and prioritize them. Field data inform us on where to act, and on what – along all events that shape a contemporary chemical economy.

7.03.T-02 Determining the Fraction of Regional Carrying Capacities Occupied by Pesticide Use

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Chemical pollution can exert adverse effects on ecosystems. To determine the safe operating space for chemicals, methods are needed to both calculate and link the actual chemical pressure on ecosystems (as a result of chemical usage) and the capacity for ecosystems to withstand that pressure without irreversible adverse effect (the carrying capacity). However, challenges in developing these methods include variability in where chemicals are used, and spatial differences in features of ecosystems (e.g., location of water bodies). Additionally, regions with seemingly distinct ecosystem carrying capacities may be interconnected as chemicals emitted into one region may occupy the safe operating space in other (e.g., downstream) regions. To address this gap, we introduce a spatially explicit framework to relate chemical pressure to region-specific carrying capacities. We quantify the fraction of ecosystem-specific carrying capacities that is occupied (and potentially exceeded) by toxicity-related pressure from chemicals. As a demonstration of this approach, pesticide application data for 409 chemicals at the county level for the United States (US) were collected from the US Geological Survey, and were used as proxies for emissions to agricultural soil. These data were integrated into the Pangea spatial multi-scale model to determine the freshwater concentration of each chemical in 45,576 different catchments in the US. Chemical pressure and carrying capacity were combined, so the final output for each chemical in a region is the fraction of a freshwater ecosystem's carrying capacity occupied by that chemical, $F_{cc,o}$ (considering both direct and indirect contributions into that region). For each region, the $F_{cc,o}$ can be analysed per chemical (e.g., 6×10^{-4} of catchment 20819's carrying capacity is occupied by atrazine in Scott County, Missouri) and across chemicals (6.6×10^{-4} of catchment 20819's carrying capacity is occupied by 10 pesticides) to assess cumulative chemical impacts on ecosystems. Results indicate that pesticide pressure varies widely across both pesticides (e.g., due to differences in chemical persistence) and catchments (e.g., due to differences in available water volume). This framework can be used to assess the pressure of current chemical emissions on ecosystems with consideration of spatial variability to meet the chemical pollution-related targets of the EU Chemicals Strategy for Sustainability and the European Green Deal.

7.03.T-03 Prioritizing Hazardous Chemicals in Food Contact Materials

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Over 12,000 chemicals are possibly used in the manufacture of food contact materials (FCMs), and several thousand chemicals have been detected in the extracts or migrates of FCMs. By transferring into food, these chemicals become directly relevant for human exposure. Concepts like the 'do no harm' principle have been adopted within the European Union's Green Deal and Chemicals Strategy for Sustainability (CSS), implying the need to identify hazardous chemicals, including those in FCMs. However, an easily accessible overview of chemicals of concern used to manufacture FCMs is missing, although this would be important for policymakers and other stakeholders. Therefore, we compiled a list of food contact chemicals of concern (FCCoC) by cross-checking databases on chemicals used in FCMs against publicly available hazard properties mentioned in the CSS such as being carcinogenic, mutagenic, or toxic to reproduction (CMRs), endocrine-disrupting chemicals (EDCs), and chemicals with persistence-related hazards. For each FCCoC, we compiled data describing the material types it is potentially used within, its hazard properties, production volume, and whether there is empirical evidence for migration and/or extraction from FCMs. We identified 388 FCCoC that have hazard properties mentioned in the CSS. The data show that the manufacturing of plastics, paper & board, and printing inks potentially use more than half of these chemicals. Moreover, around one-third of these FCCoC have been detected in migrates or extracts of at least one FCM type, including roughly 100 CMRs and 14 EDCs. This demonstrates that a large number of chemicals known to be hazardous is present in different types of FCMs and are relevant for human exposure. Taken together, we present an approach for how to identify chemicals in food packaging that could pose the greatest risk to human health. The compiled list of FCCoC can help to prioritize substances that are of particular concern for further in-depth assessment and regulatory action in frame of the CSS. Thus, our data can contribute to the safe and sustainable use of chemicals.

7.03.T-04 First Steps Toward Sustainable Circular Uses of Chemicals: Advancing the Assessment and Management Paradigm

Zhanyun Wang and Stefanie Hellweg, ETH Zurich, Switzerland

Environmental and human health impacts associated with chemical production and losses from value chains make the current

linear produce-use-dispose model no longer an option for chemicals. In this study, we explore next steps on how to embed the concept of “sustainable circularity” into practice (including the design phase) to foster systemic transition toward sustainable circular uses of chemicals. We first analyze major causes of chemical losses throughout their life cycles. Then, we propose to advance the current chemicals assessment and management paradigm by introducing (1) the consideration of multiple use cycles in the hazard and risk assessment stage and (2) an additional “sustainable circularity” assessment stage subsequent to the hazard and risk assessment stage, as a critical first step to guide systematic decision-making at all levels toward sustainable circular use of chemicals. We discuss how these new assessment stages may look like, and then we further look into how to enable the proposed changes and a larger systemic transition, both on the technical and socioeconomic sides.

7.03 How to assess sustainability of chemicals and operationalize 'safe operating space for chemicals', 'do no harm' principle, and 'safe and sustainable by design' concepts? (Virtual Only)

7.03.V-01 Retrospective Analysis of Environmental Classification & Labelling Under CLP Regulation (EC) No 1272/2008

Madeline Carsique¹, Flavio Marchetto², Marco Mattiuzzo³, Konstantinos Prevedouros³, Marta Sobanska⁴, Simon Uphill⁵ and Stella Jones³, (1)ANSES, France, (2)ICPS, Finland, (3)European Chemicals Agency (ECHA), Finland, (4)European Chemical Agency, Helsinki, Finland, (5)European Chemical Agency (ECHA), Finland

The widespread use of chemicals often comes along with harmful effects to humans and the environment. The Classification, Labelling and Packaging (CLP) Regulation (EC, No 1272/2008) obliges actors in the supply chain to appropriately classify, label and package hazardous chemicals. It is a solely hazard based process which uses the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as a basis for hazard assessment within the EU. Regarding the classification for environmental hazards, CLP focuses on the aquatic compartment, notably on the results of aquatic toxicity tests performed on fish, aquatic invertebrates and algae or other aquatic plants, which are considered as surrogates for all aquatic organisms. During the hazard assessment of a substance, its degradation and bioaccumulation behaviour are also taken into account as elements of long-term hazard assessment. In general, the hazard assessment is based on comparisons of available data with the CLP criteria. Harmonised classification and labelling (CLH) is the regulatory mechanism assuring the consistent and scientifically sound hazard identification and communication. It addresses hazards of the highest concerns such as carcinogenicity, mutagenicity, reproductive toxicity (CMR), respiratory sensitizers and other hazards on a case-by-case basis. All substances with harmonised classifications are listed in Annex VI of CLP which triggers various obligations for manufacturers, importers, and professional users of those substances. The objective of the current analysis is to present a historical perspective of the harmonised classification process under CLP for environmental hazards and an in-depth analysis of the key information since the adoption of CLP in 2008. It presents the overall statistics of the process until August 2021, including total numbers of substances classified for environmental hazards, types and severity of adopted classifications, numbers of new or updated classifications, analyses of the involved actors, classifications per substance function, species sensitivity, etc. It further explores the effectiveness of the CLH process as the regulatory risk management tool by analysing implications under the current regulatory system as opposed to the previous system. It is hoped the analysis will facilitate regulatory authorities in evaluating the hazards to aquatic environment and to assist authorities in a consistent decision-making process.

7.03.V-02 Sustainability Index - a Chemical Sustainability Metric

Hans Plugge, Safer Chemical Analytics LLC, United States

Sustainability of chemical compounds is often ill described (if at all). To truly green our chemical footprint we first must quantify the environmental and health footprint of chemical enterprises. Most chemicals in commerce are chemical products i.e., mixtures (even so-called pure chemicals as technical grades often contain up to 5% impurities.) Hazard and risk assessment methods for mixtures are few and far between. How then to assess the greening of an enterprise that distributes dozens or even thousands of products? As part of the ESG dynamics, chemical sustainability i.e., the impact from chemicals and how to measure these across hundreds or more products, is a daunting, but necessary task. We need an index that tracks the potential risk of an enterprise's total inventory as it greens over the coming years. Note here the dichotomy between risk and hazard, where environmental health measures risk as the product of hazard and exposure, i.e. quantity matters (most systems consider only hazard i.e. don't distinguish between test tubes and tanker trailers). Our Sustainability Index (SI) measures both hazard and risk across numerous chemicals – software being the only limitation. The resulting SI's for single chemical and facilities are expressed such that they can be summed across chemicals and indeed facilities to wrap up into a single corporate SI. But what about growth – as long as growth occurs in greener chemical inventory, a company can both grow and improve its SI. Given the lead-time for developing true green alternatives to existing chemicals, intermediate substitution of less hazardous “analogues” may drive initial SI reduction. Exposure here is measured as the total of chemical X within a facility. The hazard portion is less straightforward – although many hazard assessment/ranking systems exist, they generally do not allow compounding effects into a single score. There is however a hazard assessment system that is globally embraced: the so-called GHS (Globally Harmonized System) chemical classification system. We developed a metric based on the various endpoint ratings. Such metrics are then pooled across endpoints using a weighting system to result in a single hazard score for each chemical. Facility SI's can thus be established by simple summing of chemical specific risk estimates. A next step in the process would be to incorporate LCA elements into the SI.

7.03 How to assess sustainability of chemicals and operationalize 'safe operating space for chemicals', 'do no harm' principle, and 'safe and sustainable by design' concepts? (Poster)

7.03.P-Mo280 Environmental Impact Reduction of Crop Protection - How to Model, How to Measure, How to Achieve?

Anja Gladbach¹, Yuyue Zhang², Peter Fantke³, Luke Settles⁴ and Daniel Glas¹, (1)Bayer Crop Science, Germany, (2)DTU (Technical University of Denmark), Denmark, (3)Technical University of Denmark, Denmark, (4)Bayer Crop Science, United States

Agricultural activity has always had an impact on the environment. Efforts to make fields more productive and increase the efficient use of land and resources include tools like the use of pesticides to protect crops from (yield) losses due to weeds, diseases, and insect damage. Each tool that a farmer applies has benefits and drawbacks, and this is generally true for crop protection. And while crop protection products are not only developed to achieve efficacy, but as well rigorously tested to be safe for people and to pose no unacceptable impact on the environment, they still have an effect. Bayer thus in 2019 committed to reduce the environmental impact of our agrochemical uses by 30% by 2030. Reducing the environmental impacts of agrochemicals requires a coherent and efficient metrics system that allows a standardized quantification of the level of sustainable measures implemented and the extent of the associated impact. Therefore, impact indicators within a shared framework of regulation, practice, verification, and certification are needed to drive change. Based on a comprehensive and spatially resolved application dataset for >90% of the global crop protection market we quantified pesticide emissions and ecotoxicity impact, building on PestLCI and USEtox as state-of-the-art, consensus-based models for application in life cycle assessment (LCA) and environmental footprinting. After initial identification of global hotspots and drivers, we will assess the effects of reduction levers (e.g., emission management and mitigation, precision application, combination of chemical and biological crop protection, substituting high-impact chemicals by functionally equivalent more sustainable alternatives). We will use the results to achieve impact reduction ambitions and to guide company efforts within the safe and sustainable-by-design framework for crop protection. In this talk, we not only want to share how we approached this commitment (eg. identification and selection criteria of suitable models, setting of a reliable baseline scenario, associated data needed and the evaluation of the suitability of related impact reduction options), but as well the limitations, challenges, and learnings so far. With the proposed assessment framework, we aim to contribute to the discussion on how to achieve environmental impact reduction for agricultural production as well as to outline current limitations like data availability or incentivization systems for farmers.

7.03.P-Mo281 Framework for Evaluating Emerging Chemical Issues for Prioritising Management

Olivia Lin Tran¹, Graham Merrington², Adam Peters³, Therese Manning⁴, Anna Ramarosandratana⁵, Julie Cattle⁶ and Janina Beyer⁷, (1)wca, Faringdon, United Kingdom, (2)WCA Environment Limited, United Kingdom, (3)wca, United Kingdom, (4)Enriska, Australia, (5)NSW Environmental Protection Authority, Australia, (6)NSW Environment Protection Authority, Australia, (7)NSW DPE, Australia

Regulatory organisations operate within budget and resource constraints, whilst having increasing responsibilities to protect human health and the environment with evidence-based actions. This poster will provide a simple, intuitive, evidence-driven framework to support the identification of chemical issues to assist regulators target their resources to reduce potential human and environmental risks from chemicals. The framework can be used to support the evaluation of chemical issues at varying organisational levels (e.g. global, national or state-level) and help regulators understand what chemical issues and risk management options are within their departmental remit. A critical part of this framework is defining the chemical issue to give a workable scope for evaluation and targeted intervention. Chemical issues are identified where there is a source, pathway and receptor linking a chemical to an adverse or undesirable outcome. It is not simply a chemical or group of chemicals but must have an evidenced source, pathway and receptor. It is critical that the identification of chemical issues is supported by robust evidence in the source-pathway-receptor framework to justify regulatory intervention to mitigate risk. Assessment of issues for further risk management can then take place considering the strength of evidence in each group, the linkages between them and the local and/or regulatory context. Equally, gaps in the evidence can act as a critical driver for targeted studies and monitoring by both the regulator and the scientific community. An Excel-based tool has been developed to capture the range of chemical issues under consideration for assessment. Issues can be filtered (using standard category labels under each of the groups) to determine frequently occurring categories for evaluation, if any interventions can be made to target multiple issues having the same categories. Additional information, such as spatial and temporal scope, economic and social impacts and other non-chemical factors contributing to the issue can be captured and considered in the assessment process. Example evaluations will be presented to illustrate the assessment process and next steps for risk intervention or information gathering.

7.03.P-Mo282 Polymeric and Monomeric Brominated Flame Retardants: Degradation Behaviour and Ecotoxicological Effects

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Conventional brominated flame retardants (BFRs) are widely used and have been subject to thorough scientific investigation and regulatory attention due to concerns about their behaviour in the environment. Thus, in recent years the development of novel brominated flame retardants has been intensive, while the research on these compounds lags behind. Improvements of the environmental behavior are commonly stated as the reasons for the development of such novel BFRs. To evaluate the success of these substances, more studies are needed on characteristics such as the bioaccumulation, mobility, degradation or toxicity of novel BFRs. One example of a BFR explicitly designed for greater sustainability is PolymericFR, a brominated copolymer whose large structure leads to a decreased mobility and bioavailability when compared to conventional BFRs. However, it has been shown that abiotic degradation processes result in the formation of smaller, more mobile degradation products of this polymeric BFR, facilitating releases by the chemical at the end-of-life phase. Therefore, in this study we examine the photolytic degradation of PolymericFR and compare it to the degradation of the monomeric alternative BFR Tetrabromobisphenol A-bis(2,3-dibromo-2-

methyl-propylether) (TBBPA-Ether). Our methods combine chemical analysis and ecotoxicological testing of photolytic degradation mixtures derived under various conditions. Both BFRs were irradiated in an aqueous environment for a range of time intervals, simulating the UV exposure of the compounds for up to nine days in sunlight. The resulting degradation mixtures were filtered, stored and subsequently applied in further analysis. The analyses included high-resolution mass spectrometry (LC-HRMS) and ecotoxicological tests with *Desmodesmus subspicatus*, *Daphnia magna* and *Lumbriculus variegatus*. Depending on the test organisms, acute effects (*D. subspicatus* and *D. magna*) or chronic effects and physiological reactions (*L. variegatus*) were studied as ecotoxicological endpoints for the testing. Where possible, median effective concentrations (EC₅₀ values) were calculated and compared for the different degradation conditions and the two BFRs. The newest results of our evaluation will be presented and discussed. Overall, our study deepens the current understanding on the degradation behavior of two novel BFRs and exemplifies the relevance of degradation and transformation processes in ecotoxicology.

7.03.P-Mo283 Multi-Criteria Assessment of Pesticide Use, Ecotoxicological Risks, Ecological and Economic Impacts of Swiss Apple Production

Marcel Mathis¹, Judith F. Blom², Thomas Nemecek¹, Esther Bravin¹, Philippe Jeanneret¹, Otto Daniel¹ and Laura De Baan¹, (1)Agroscope, Switzerland, (2)Plant Protection Products - Impact and Assessment, Agroscope, Switzerland

The European Commission's «Sustainable Use of Pesticides» Directive aims to better protect human health and the environment by reducing the risks and impacts of pesticide use and promoting lower-risk alternative approaches or techniques. Such alternatives may include the use of less hazardous pesticides or non-chemical alternatives such as mechanical techniques or biocontrol measures. However, these alternatives are not always equally effective and may finally result in yield losses. They also require additional material, energy or labor inputs, which can lead to additional undesirable environmental or economic impacts. Understanding potential trade-offs is crucial to initiate a sustainable pesticide use. In this study, we have analysed such trade-offs using apple production in Switzerland as an example. Apples are very susceptible to diseases and pests and are currently among the fruit with the highest intensity of pesticide use. We conducted a multi-criteria assessment of exemplary crop protection strategies, including different chemical and non-chemical alternatives to standard pesticides, in different apple production systems (conventional, integrated, and organic). An assessment of pesticide use, local ecotoxicological risks to next-to-field habitats, global environmental impacts (life cycle assessment) and an economic analysis was performed based on 13 quantitative indicators. Compared to the standard crop protection strategy, the integrated strategy with reduced pesticide use performed better in terms of ecotoxicological risks and biodiversity, but was not profitable due to high investments, resulting in negative hourly wages for farmers. The organic strategy showed higher impacts per kg of apples produced for most global environmental indicators, but performed better for ecotoxicological risks, biodiversity and also farmer's hourly wage. The conventional strategy performed worse in terms of biodiversity, global warming potential and investment costs, but resulted in higher hourly wages for farmers. This study shows that reducing pesticide use and the associated ecotoxicological risks comes with trade-offs in other environmental aspects and can be an economic challenge. None of the strategies examined performed better than the others in all the 13 indicators assessed. However, our comprehensive approach can help develop more sustainable crop protection systems and evaluate alternatives to pesticide use from an environmental and economic perspective.

Track 8: Special Sessions

8.01 Chemicals and Sustainability

Special session with no abstracts.

8.02 Going MAD – Communication and goals of mechanistic effect models in regulatory ERA

Special session with no abstracts.

8.03 The European Green Deal (Chemicals Strategy for Sustainability)

Special session with no abstracts.

8.04 Extended submission (Virtual Only)

8.04.V-01 A Comparison of the Variability Across Bumblebee Colony Parameters Using Manual and Automated Methods: How Accurately Can We Measure Bombus Colony Development?

Maryam Qureshi¹, Lukas Materne², Catherine Borrek¹, Nina Exeler¹, Frederic Tausch², Klaus Thoiss¹, Katharina Schmidt², Peter Trodtfeld¹ and Maria Teresa Almanza¹, (1)Bayer Crop Science, Germany, (2)apic.ai, Germany

Bumblebees are important wild and managed pollinators of agricultural crops and other plants. Some bumblebee species can be reared, and their colonies are commercially available. Thus, testing material for ecotoxicological studies is readily available. Recently validated testing methods have been developed for individual adult bumblebees (OECD 246/247) but the development of higher tier studies that would allow the assessment of colony development has proven to be more challenging. In situ-evaluation of colony development in higher-tier testing approaches would be crucial, since existing data reveal a very high inter-colony variability, even under identical test and exposure conditions. Various approaches have been developed with some success to overcome this issue. Yet, it is still technically challenging to accurately measure parameters such as colony strength, individual's mortality and size and foraging activity at regular intervals in the field without disturbing colony development. Therefore, we have jointly been developing an approach to compare "conventional" assessment methods like manual counting of

individual bumblebees or estimation of brood areas, and novel automated, sensor-based methodologies to survey these parameters. The conventional methodologies are time-consuming, and their accuracy can be compromised by the limited frequency and duration with which they can be conducted due to technical constraints. The automated sensor-based methodologies, in contrast, may offer a more efficient and more accurate option. In this work, we are presenting the comparison of conventional and sensor-based measurements done in parallel in an exemplary trial on 6 colonies per treatment group (control and 2 concentrations of a toxic reference) for different parameters. Our work contributes to a better understanding of between-hive variability in bumblebees, and the influence of different assessment methods to the outcome of the measurements.

8.04.V-02 A Sustainable Solution for the Use of Leaching Agents in the Indirect Mineral Carbonation

Yoonah Jeong¹, Ye-Eun Lee² and I-Tae Kim², (1)KICT, Korea, South, (2)Korea Institute of Civil Engineering and Building Technology (KICT), Korea, South

Carbon capture, utilization, and storage (CCUS) is widely used to reduce CO₂ emissions and handle industrial solid waste. Indirect carbonation is one of the CCUS techniques, which convert gaseous CO₂ into thermodynamically stable carbonates form by using alkaline oxides or hydroxides. Although indirect carbonation is efficient as well as reliable approach to store CO₂, sustainability issues arise due to the use of various leaching agents. Indirect carbonation needs the elution of alkali earth metals (Ca and Mg) from raw material using various solvents, which leads to the use of a high amount of chemicals and the generation of chemical wastes. Therefore, this study aims to derive a sustainable solution for the process of indirect mineral carbonation. We investigated the various leaching agents and concentration settings to find an optimized process of indirect carbonation while producing carbonates as a final product.

8.04.V-03 Advancing the In Situ Toxicity Identification Evaluation (iTIE) Technology for Identifying Toxicants

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The iTIE is being optimized to: (1) improve use in sediments via developing diverless deployment modifications, (2) modifying the water intake system for porewater extractions, (3) developing a gentle aeration system to allow for toxicity testing of anoxic pore and surface waters, (4) refining chemical targeting (including PFAS) and separation via new resins, (5) expand marine and freshwater fish and invertebrate chronic toxicity, and (6) further field validate the usefulness of the technology. The iTIE system (iTIES) is a biological fractionation protocol that systematically identifies chemical classes causing toxicity in overlying water, pore water, and outfall effluents (i.e., industrial/municipal point source and stormwater). The system separates chemical classes of contaminants of concern (i.e., various types of organics, metals, ammonia), frequently linked to adverse biological effects. The iTIES prototype 3 is now a robust deployable system that allows for consistent and sensitive adjustments to pumping rates of ambient waters through a diagnostic array of resin treatments. The current battery of resins separates the following potential toxicants: ammonia, problematic heavy metals (Ag, Cd, Cu, Ni, Pb, Zn), and organics of various characteristics, including PCBs, PAHs, and PFAS. It provides a unique diagnostic tool for used in a tiered risk assessment. The iTIES diagnostic tool is to be used once Tier 1 assessments suggest chemical toxicity may be a concern.

8.04.V-04 Approach to Inform About the Representativeness of Climatic Conditions During Foliage RESIDUE Decline Studies

Joachim Nopper¹, Tim Haering¹, Eduard Szöcs¹ and Kai Ristau², (1)BASF SE, Germany, (2)APD/EE, BASF SE, Germany

In the wildlife risk assessment for the registration of plant protection products, foliage residue decline (DT50) studies are often used as a higher-tier refinement for providing more realistic estimates of the residue decline of a plant protection product on the herbivorous portion of an animal's diet. In EFSA's draft of the updated birds and mammals guidance document from 2021 a mandatory relevance and reliability check of this study type is proposed. One critical element of this assessment is the determination of the representativeness of the locations of residue decline studies for the geographical region which is specified in the GAP. To facilitate this check, we present an approach that is based on publicly available spatial data for cumulative precipitation, average air temperature and land use for informing about the representativeness of the climatic conditions at the trial site during the study period. Weather data measured at the trial site, namely cumulative precipitation and average daily temperature, are compared with reference data from the last decade provided at a 0.31x0.31 degrees grid resolution for the European Union. Spatio-temporal buffers can be also included to have a greater extent of reference values. Agricultural production can act as a filter to exclude references from the comparison. Simple graphical representations and statistics of the representativeness check are proposed.

8.04.V-05 Assessment of Cement Production on Water Quality in Sagamu, Ogun State, Nigeria

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The contamination of groundwater by organic and inorganic compounds are severe worldwide problems because groundwater serves as a universal source of water. Contaminated water is unsuitable for various purposes and can be harmful for human health and the environment. This study aimed at determining water quality parameters in borehole water around Lafarge cement factory, Sagamu, Ogun State. Water samples were collected from twenty residential houses in all four cardinal locations around the factory while distilled water served as control. Physicochemical parameters such as pH, Turbidity, salinity, chloride, sulphate, nitrate and phosphate were determined using standard method of analysis, while heavy metals such as calcium, magnesium, lead, cadmium, nickel, iron, copper, and zinc were determined using Flame Atomic Absorption Spectrophotometer (FAAS). Data were

analyzed using statistical techniques (mean±standard deviation), Pearson product moment correlation at 5% ($P < 0.05$) level of significance and ANOVA. Physicochemical parameters results were obtained in the following range; pH (7.71-8.07), salinity (0.11–0.43 µg/L), turbidity (6.5–7.74 NTU), conductivity (230-460µohms/cm), phosphate (0.01-0.018), magnesium (0.03–0.13 mg/L) calcium (9.18–30.4 mg/L), chloride (10.4-75.4 mg/L), and nitrate (3.01-4.89 mg/L). Values of pH, conductivity, chloride and phosphate were all below the permissible limits of the WHO standard. Nitrate and turbidity were above the WHO permissible limits. The concentration of heavy metals (mg/L) were in the following range; Fe (0.005-0.507), Pb (0.012-1.33), Cu (0.001-0.014), Cd (0.002-0.005), Ni (0.006-0.008), and Zn (0.003-0.258). The average concentration of lead (0.2567±0.34 mg/L), and cadmium (0.0036±0.0007 mg/L) exceeded WHO guideline for lead (0.01) and cadmium (0.003) in water. A significant correlation existed only between Cd and Fe ($p < 0.005$). ANOVA showed no significant variations ($p < 0.05$) among the different sampling locations. Risk estimation analysis indicated that consumption of 2.567 litres of water from this area exposes inhabitants to the risk of lead toxicity, consumption of 0.36 litres of water exposes them to cadmium toxicity. Pollution Index (PI) value greater than 1.0 was obtained for lead (25.7 Pi), nitrate (4.85 Pi), turbidity (1.44 Pi), cadmium (1.17 Pi) and pH (1.05 Pi), which shows significant degree of pollution. Borehole water quality from this location is compromised and vulnerable to pollution, hence, it is important to treat it before consumption.

8.04.V-06 Big Brother.....in the Sediment: First Application of the Multispecies Freshwater Biomonitor in Sediment and Soil

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Behavioural endpoints are amongst the most sensitive test parameters of sublethal ecotoxicology, often linked to both biochemical biomarker responses at the molecular level and to ecological consequences for the population at higher organization levels. Non-optical quantitative behavioural recording allows to monitor the animals (individual or in groups) also in soil, sludge and sediments, where visual methods fail. See here the 1st results of the application of the Multispecies Freshwater Biomonitor (MFB), based on the non-optical quadrupole impedance technology, using different aquatic benthic invertebrate species and sediments.

1) Firstly, quantitative behavioural signals were generated equally in water, sludge, soil and sediment: For example, in freshwater sediments signals of *Chironomus* spp. and *Hydropsyche* spp. approve the method. 2) Secondly, the grain size composition of the sediments used for culture and maintenance of your test organisms might be important factors for their behavioural performance: For example, the behaviour of *Lumbriculus variegatus* was compared when reared in sediments of different grain sizes. After several weeks of exposure the medium sized grain sizes were preferred, here the animals were more active compared to coarse or fine grain sizes (1). 3) Toxicity experiments were performed to study avoidance of *Eisenia fetida*, exposed to different dilutions of sewage sludge in choice setups in the MFB over 48 hours. Increasing concentrations of sewage sludge led to increased avoidance of the worms (2). 4) The marine amphipod *Corophium volutator* was exposed to different types of toxicants, water accommodated fraction of oil and the pesticide Bioban in short term experiments in the MFB. In both cases, the activity of the amphipods increased with increasing concentrations of the toxicants (3,4). These initial studies show the potential for the non-optic behavioural recording technology of the Multispecies Freshwater Biomonitor as an appropriate tool for sediment and soil ecotoxicology in the future. References (1) Sardo, M. *et al.* (2007): 'Behavior, Growth, and Reproduction of *Lumbriculus Variegatus* (Oligochaeta) in Different Sediment Types'. Human & Ecological Risk Assessment, 13:3,519-526. (2) Rastetter, N. & A. Gerhardt (2016): Toxic potential of different types of sewage sludge as fertiliser in agriculture: ecotoxic. effects on aquatic, sediment and soil indicator species. J Soil & Sed., 18 pp. (3) Kienle, C. & A. Gerhardt (2008): Behavior of *C. volutator* (Crustacea) exposed to the water accommodated fraction of oil in water and sediment. Envir. Toxicol. Chem. 17,3: 599-604. (4) Kirkpatrick, A. *et al.* (2006): Use of the multispecies freshwater biomonitor to assess behavioral changes of *Corophium volutator* (Pallas, 1766) (Crustacea, Amphipoda) in response to toxicant exposure in sediment Ecotox., Environm. Saf. 64, 298-303.

8.04.V-07 Cumulative Chronic Exposure Assessment of Carbamate and Pyrethroid Pesticides in Thai Population Through Commonly-Consumed Vegetable Consumption

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Consumers could expose to more than one pesticide at a time, and the risk of adverse health effects would be increased if the pesticides share the same mechanism of toxicity or have the same mode of action affecting the same target organ. Carbamate (CB) are compounds widely used in agriculture and exhibit neurotoxicity via inhibition of the acetylcholinesterase enzyme in the nervous system. Pyrethroids (PYR) are used as agricultural and household insecticides and interact with voltage-gated sodium channels, resulting in disruption of membrane excitability in the nervous system. This study aims to estimate the risk of chronic exposure to CB and PYR cumulative dietary exposure of 7 population subgroups of Thai consumers from vegetable consumption. The cumulative dietary CB and PYR exposure was estimated using a deterministic approach and considered pesticide residue and food consumption data. Pesticide residue data were obtained from a peer-reviewed study by Wanwimolruk *et al.* 2016. Food consumption data were obtained from a validated questionnaire-based national dietary survey from Mahidol University published in 2016. Three commonly-consumed vegetables were included in the assessment: Chinese kale, pak choi, and water morning glory with a total of 397 samples. The relative potency factor (RPFs) approach was used to estimate cumulative dietary exposure using oxamyl and deltamethrin as Index Compound (IC) for CB and PYR, respectively. The RPF were derived from BMD₁₀ or NOAEL to solve the CB and PYR data availability. The chronic cumulative dietary CB and PYR exposure estimates at 97.5th do not exceed the reference dose (RfD) of the IC of Oxamyl and Deltamethrin, respectively. Children between 3 - 6 years old were the most CB and PYR exposed population; however, the hazard quotients of exposures to CB and PYR were less than 1 % of IC RfD. The major contribution to the chronic CB exposure for the Thai population subgroups was made by morning glory which

ranged between 49% to 61% additionally, the pesticide that contributes the most was carbaryl. While the main contribution to the chronic PYR exposure for the Thai population subgroups was made by Chinese kale which ranged between 66% to 79%, and cypermethrin was the major contributor. Exposure estimates suggest insignificant dietary risk from the three commonly-consumed vegetables in Thailand.

8.04.V-08 Developmental Effects of 700 and 3500 MHz Cellphone Frequencies on the Zebrafish Embryo Model

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Data integration and improvement in connectivity have been achieved by the development of the 5G telecommunication spectrum. The spectrum < 1000 MHz will be used for connection between remote areas, whereas the bands of 1000 - 6000 MHz and above will improve connectivity to individual devices and small zones. In order to assess potential health effects, and developmental effects due to these radiofrequencies we have exposed zebrafish embryos to 700 MHz and 3500 MHz (~ 45 V/m) at the beginning of their development for 1 and 4 h. Toxicity endpoints were evaluated up to 120 hpf. Exposure was performed in a controlled exposure chamber GTEM ETS-LINDGREN 5402 coupled with a Signal Generator (250 kHz-6000 MHz) that produces pure sinusoidal signals at the stated frequencies with required power during the exposure. The generator is connected to an amplifier ZHL-42W (10 MHz-4200 MHz). Zebrafish (96 embryos/treatment) were located in an area of maximum field uniformity. Control embryos were located outside the chamber and were evaluated for background exposition by means of the MVG EME SPY Evolution Dosimeter. Temperature was maintained at 25°C both inside and outside the GTEM. Results show no effect on mortality or heart rate with exposure. However, neurodevelopmental effects were observed. Exposed animals were hypoactive both at the 24 hpf tail coiling assay and the 120 hpf visual motor response test, with stronger effects at 700 MHz. In addition, decreased stress response was observed in larvae exposed to 700 MHz. Neurodevelopmental effects are the most probable cause of behavior alterations by exposure to these radiofrequencies. In this study, we have demonstrated a valid and robust setup for biological model exposure to radiofrequencies. Future studies will include exposition to other wavelengths in the 5G technology, lower levels of signals associated to environment exposition and increased exposure time.

8.04.V-09 Eco-Toxicity of Northern Ontario Petroleum Hydrocarbon Contamination Using Native Boreal Plants and Invertebrates

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Petroleum hydrocarbons (PHCs) are a class of organic pollutants that may pose environmental risks if left unaddressed. They can be toxic to numerous organisms including plants, invertebrates, amphibians, reptiles, and mammals. Soils collected from a site in northern Ontario were solvent extracted using 1:1 acetone/hexane to reveal 16,000+ ppm (F2: 10300 ppm; F3: 4960 ppm; F4: 1440 ppm) total petroleum hydrocarbons. The F2 and F3 concentrations exceed both provincial guidelines outlined in the Canadian Environmental Protection Act (2011) and Canada-Wide Standards outlined by the Canadian Council of Ministers of the Environment (2001). To derive site-specific environmental guidelines, a series of toxicity experiments were carried out using the procedure provided in Environment Canada's Biological Test Method: Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region (2013). Briefly, five plant species were grown from seed in multi-concentration laboratory mixtures of contaminated and control (uncontaminated) soil from the site: i) *Elymus trachycaulus*, ii) *Achillea millefolium*, iii) *Picea mariana*, iv) *Salix bebbiana*, and v) *Alnus viridis*. The experiments revealed that *P. mariana* and *A. millefolium* had the highest germination success and growth of shoot and root endpoints in the contaminated soil (IC₅₀: 25% - 62%), while the germination (chi-square test; p=0.004) and growth of *S. bebbiana* (IC₅₀: 8.7% - 22%) was significantly impaired relative to the control soil. Reference tests using boric acid as a reference toxicant revealed high seed quality and sensitivity to soil contaminants at the time of the toxicity tests. Survival and reproduction tests using age-synchronized native *Folsomia candida* and *Proisotoma minuta* springtails in accordance with standardized testing methods outlined in Environment Canada's Biological Test Method: Test for Measuring Survival and Reproduction and Springtails Exposed to Contaminated Soil (2014) will also be reported on.

8.04.V-10 Ecotoxicological Evaluation of Engineered Biochars

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Biochar is a solid material produced by the carbonization of biomass in the pyrolysis process. In recent years biochar gained attention in various areas of life. Type of biomass, pretreatment method, and pyrolysis condition (temperature, duration, carrier gas) affect physicochemical properties of biochars. Although biochar properly fulfils its functions generally, in some cases it needs modifications to enhance effectiveness. One of the methods of biochar modification is impregnation or coating the surface of the biochar with metal oxides. The metal modification enhances biochar sorption capacity to heavy metal elements, nutrients, and organic pollutants. As many works were published recently considering pristine biochar toxicity, there is a knowledge gap regarding the safeness of modified biochars to different organisms. In this work, three ecotoxicological tests were applied to determine the toxicity of engineered biochars to bacteria (*Aliivibrio fischeri*) and invertebrates (*Folsomia candida* and *Daphnia magna*). Willow or sewage sludge were impregnated with Zn or Mg and pyrolysed at 500 or 700°C. The content of toxic substances (e.g., polycyclic aromatic hydrocarbons and heavy metals) in biochars and engendered biochar was determined. Metal modification resulted in lower polycyclic aromatic hydrocarbons content compared to pristine biochar. Heavy metal content in biochars and their pH affected ecotoxicological properties of biochars. A dependence on the applied dose of biochar was also observed. Obtained results can be useful for finding solutions for safe biochar application, compatible with a circular economy and the requirements set by the European Union.

8.04.V-12 Effects of Pesticides Combination on the Development of Stingless Bees *Scaptotrigona postica*

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Stingless bees are important pollinators of natural areas and agricultural crops, due to their crucial role in maintaining biodiversity and economics. During foraging the bees are exposed to different pesticides which can have direct action on them and in colony health, once collected resources may contain pesticide residues that will be taken inside and used to feed larvae and adults. Many studies have demonstrated the effects of different pesticides on bees, but studies using combinations of molecules are still scarce/limited, especially with stingless bees, which is closer to the reality of the field conditions and Brazil. In this way, we provide possible effects of residual field doses of the insecticide imidacloprid and the herbicide glyphosate on the development of stingless bees *Scaptotrigona postica*, *in vitro*. The 1st instar larvae subjected to acute exposure larval food containing pesticides at the following concentrations: (a) control (without addition of pesticides), (b) 0.02 µg/µL of glyphosate, (c) 0.02 µg/µL of imidacloprid and (d) 0.02 µg/µL of imidacloprid + glyphosate. Effects on mortality, pupation, emergence rate, and survival were evaluated. We also evaluated the head width and intertegular span morphometry of newly emerged bees. In the bioassays, no rejection of the diets with pesticides by the larvae was observed. The exposure of the larvae to pesticides did not affect the defecation rate or development time. Experiments with the diets showed a mortality rate of (a) 0%, (b) 3%, (c) 30% and (d) 13%, where the diet (c) there was a significant increase ($p > 0.05$) in the mortality rate when compared to the control. When intertegular span distance and head width measurements were evaluated, bees exposed to diets (b) and (d) showed significantly higher measurements when compared to the control. This study demonstrates that chronic exposure of stingless bees to glyphosate and imidacloprid can have effects on colony survival, asymmetries in their body structure that result in maladjustments in the bees' flight ability and even disability for flight. FAPESP 2017/21097-3; CNPq 400540/2018-5.

8.04.V-13 Evaluation of Existing Models for Estimating the Bioaccumulation of Pesticides in Earthworms Using a Large Experimental Dataset

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Pesticides can accumulate in earthworms and exert adverse effects on earthworms and top predators (Van Hoesel *et al.*, 2017; Reed *et al.*, 2016; Reinecke and Reinecke, 2007). Standardized laboratory procedures for determining the bioaccumulation of contaminants into earthworms such as the OECD guideline 317 (OECD 2010) are very labour intensive and time-consuming. There is an ongoing community effort (see 'Mind the gap – on the way to a spatial and temporal explicit TKTD model for earthworms'; Roeben 2019) to produce a spatially and temporally explicit toxicokinetic-toxicodynamic (TKTD) model for the uptake of pesticides by earthworms. Several predictive models, including empirical and dynamic mechanistic models (Torralba-Sanchez, 2016; Jager, 1998; Belfroid *et al.*, 1993; Connell and Markwell, 1990; Armitage and Gobas, 2007; Jager, 2004; Jager *et al.*, 2003) have been developed during the last three decades to predict the bioconcentration or bioaccumulation of organic chemicals in earthworms. However, the applicability of these models in predicting body residues for a variety of pesticides and earthworm species in various soil environments is uncertain, in part due to the lack of independent data sets to test the models. We are currently generating kinetic uptake and elimination data, for five pesticides (lenacil, flutriafol, dieldrin, hexachlorobenzene and p,p'-DDT) with varying K_d values (0.2-3.4 L/kg, 0.7-10.7 L/kg, 8.9-145.4 L/kg, 178.8-2929 L/kg and 2097-34346 L/kg, respectively) by the earthworm *Eisenia fetida*, *Aporrectodea caliginosa* and *Lumbricus terrestris* in five contrasting soils. Here we present the *L. terrestris* data. The data are used to evaluate and refine existing models and identify chemical and soil properties influencing earthworm uptake. Refined models could be highly beneficial for environmental risk assessment of pesticides and provide a rapid solution for regulators to support environmental decision-making.

8.04.V-14 Exploring Methylmercury Demethylation in the Human Gut Microbiome

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Methylmercury is a potent neurotoxin that bioaccumulates in individual organisms and biomagnifies up the food chain. This toxin is found in lakes and rivers worldwide, and climate change is expected to increase its concentration in these environments. This poses a significant risk for those who rely on methylmercury-contaminated sources of predatory fish for food. Although methylmercury metabolism has been studied extensively, it remains unknown why the half-life of methylmercury can range from < 30 to >120 days in the body across individuals after ingestion. The gut microbiome is a topic of rising interest in the field of toxicology, particularly its impact on the metabolism of ingested environmental contaminants. Individuals' gut microbiomes can vary greatly depending on environmental factors, and these differences may contribute to the wide range methylmercury half-lives observed in humans. Here we present strong evidence that the human gut microbiome contributes to the metabolism/demethylation of methylmercury. Using batch incubations of healthy individuals' fecal samples with methylmercury, we observed large variations in the ability of the microbiomes to degrade/demethylate the toxin. Some microbiomes degraded virtually all methylmercury present, others only half. The degradation phenotype appears to be associated with i) fermentation capacity of the microbiome and ii) the nutrient profile of the assay medium. Alteration of metabolism with changes in nutrient profiles have implications for the effect of diet on neurotoxin transformation. This work offers a novel perspective on the role of human microbiomes in altering neurotoxin exposure and highlights the importance of gut health to an individual's overall well-being.

8.04.V-15 Honey Bee Toxicological Responses Do Not Accurately Predict Environmental Risks of Agrichemicals

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Historically, the European honey bee, *A. mellifera*, has been used as an ecotoxicology model in pesticide risk assessments and has been assumed to be a representative species to predict the risk to all other bee and invertebrate pollinator species. However, considering the vast diversity of bees, their remarkable differences in life histories, and the paucity of non-eusocial bee data, this approach could underestimate risk. To account for non-*Apis* bees in pesticide risk assessments, the European Food Safety Authority (EFSA) has proposed the solitary, cavity-nesters *Osmia bicornis* and *Osmia cornuta* as model species. However, these cavity-nesting, solitary bees do not represent 60% of the solitary bees that nest and spend their life cycle in the ground. Furthermore, the proposed model species are from the family Megachilidae, and are relatively closely related to the *Apis* bees (Apidae). The EFSA has also suggested that a 10-fold safety factor is applied to current *A. mellifera* toxicity data to estimate risk for non-*Apis* bees. In order to improve our understanding of inter-species sensitivity variability and to test if the 10-fold safety factor is sufficient, we conducted acute oral and contact bioassays of imidacloprid (neonicotinoid) and dimethoate (organophosphate) on *A. mellifera* and a New Zealand solitary, ground-nesting bee species, *Leioproctus paahaumaa*. The bees responded inconsistently: *L. paahaumaa* were 36 and 194 times more susceptible to oral and topically applied imidacloprid than *A. mellifera*, but showed equal sensitivity to dimethoate. Furthermore, the proposed safety factor of ten applied to honey bee endpoints did not cover the interspecific sensitivity difference. Additionally, OECD protocols for honey bee risk assessments were fraught with methodological issues, making standardization problematic. Our standard-setting study highlights the inadequacy of using honey bees as surrogates for all non-target species; the urgent need for more comparative inter-species toxicity studies; and the development of standardized toxicity protocols to ensure regulatory pesticide risk assessment frameworks are protective of diverse pollinators.

8.04.V-16 Investigation of Microbial Communities in Indoor Air in Multi-Use Facilities Using NGS

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Indoor air quality can significantly affect the health of residents since modern people spend more than 90% of their time indoors. The most common indoor air pollutants include biological pollutants, asbestos, particulate matter (PM), volatile organic compounds, combustion products, excessive moisture, and formaldehyde. Microbes can cause infectious diseases, asthma, allergies, etc. Some microbes are closely related to human health, such as the human microbiome. Also, the Hygiene hypothesis indicates the link between infants' exposure to environmental microorganisms and the development of the immune system. Therefore, the effects of microbial communities existing in the indoor environment on humans are known to be diverse but not clearly identified. In addition, the importance of indoor air management in multi-use facilities has increased due to the global epidemic of infectious diseases. In this study, bio-aerosol was collected using an air sampling pump and filter in multi-use facilities and measured concentration of PM using a light-scattering type dust counter. The microbial community was confirmed using a genetic analysis method. Next-Generation Sequencing (NGS) was performed to analyze the diversity of the microbial community in each sample. The composition of bacterial and fungal communities was confirmed, and dominant or keystone species were selected. Quantitative Polymerase Chain Reaction (qPCR) was performed to confirm the total amount of microbial DNA and a specific species. In addition, endotoxin and particulate matter concentration were analyzed. The correlation between various factors (room temperature, relative humidity, endotoxin, PM concentration) and diversity indices was confirmed through correlation analysis and based on results, we intend to lay the foundation for indoor air management in multi-use facilities.

8.04.V-17 Is There a Difference in the Antioxidant Defense Response of Freshwater and Marine Bivalves to Wildfire Ash-Associated Contaminants?

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Wildfires constitute a diffuse source of contamination to aquatic ecosystems mainly due to the mobilization of ash-associated hazardous substances, like metals and PAHs, by post-fire runoff. Bivalves are particularly vulnerable to ash contaminants due to their reduced mobility, filter-feeding behavior and benthic life cycle. Still, the effects of ash contaminants on this group of animals have been overlooked, especially regarding marine species. Although wildfires are more common in forest watersheds, they have been reported to affect coastal areas, with potential negative impacts for the associated biota. This study aims to assess the antioxidant defense response of freshwater (the Asian clam – *Corbicula fluminea*) and marine (the cockle – *Cerastoderma edule*) bivalves to wildfire ash. Organisms were exposed to different concentrations (0, 12.5, 25, 50 and 100%) of aqueous extracts of Eucalypt ash (AAEs) from a moderate severity wildfire. The activity of various enzymes, including catalase (CAT), glutathione peroxidase (GPx; total and selenium-dependent), glutathione reductase (GRed) and glutathione S-transferase (GST) were determined in the soft tissues of both species, as well as lipid peroxidation (by quantification of thiobarbituric acid reactive substances - TBARS) and protein content. A significant increase in the protein content of soft tissues from *C. edule* was observed for AAE concentrations >25%, whereas for *C. fluminea* no significant differences were found. Likewise, significant effects were observed on the activity of GRed and on TBARS for *C. edule*, unlike for *C. fluminea*. For both species, a significant decrease in total GPx activity was observed at AAE concentrations >25%, whereas selenium-dependent GPx activity was decreased at AAE concentrations >12.5%. The activity of CAT and GST, on the other hand, was not significantly affected by exposure to AAEs, for either species. These results suggest that *C. edule* suffered oxidative stress and consequently lipid peroxidation, after exposure to ash extracts, due to the activation of antioxidant defense mechanisms. Conversely for *C. fluminea*, the antioxidant defense mechanisms were effective in avoiding significant oxidative stress. The fact that negative effects were observed at low AAE

concentrations (?12.5%), suggests that bivalve species are highly sensitive to ash-associated contaminants, which might have repercussions for the aquatic trophic webs of both freshwater and coastal ecosystems.

8.04.V-18 Many-Mums Emissions & Fate of Organophosphate Esters From 19 Megacities Estimated Using the Multimedia Urban Model

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Organophosphate esters (OPEs), chemicals that are used as flame retardants and plasticizers, are found at relatively high levels globally in air and surface waters. Many OPEs undergo long-range transport, are persistent in the environment, and can cause adverse health impacts, leading them to be called “regrettable substitutes” for restricted flame retardants. Cities are important sources of OPEs to the surrounding environment due to high OPE production and usage. OPEs released from cities undergo transport within urban environmental compartments (e.g. air, water, soil), but most are exported to surrounding regions and ultimately remote regions via long range transport. We estimated the atmospheric emissions and fate of OPEs from 19 global “megacities”. Emissions were back-calculated using versions of the Multimedia Urban Model (MUM) parameterized for each city, based on OPE air concentrations from the Global Atmospheric Passive Sampling (GAPS) – Megacities network. Our preliminary results estimated that the 19 megacities collectively emitted ~120,000 kg yr⁻¹ of 10 OPEs in 2018. OPE fate varied substantially between cities. Per capita emissions varied from ~ 5.0 to 10,000 mg capita⁻¹ for Istanbul and London, respectively. In a model scenario using a constant air concentration across cities, cities with high rain rates, like Lagos, Bogota, and São Paulo, required emissions that were 2 – 2.5 fold higher than in drier cities like Santiago, Beijing and Cairo, to maintain the same air concentration. Under this same scenario, a larger proportion of the higher octanol-air partition coefficient chemicals accumulated in the soil and in the urban films of the “wetter” cities than in the drier cities. This led to 7.5 and 4.5 fold higher predicted concentrations in soil and film, and up to a 7-fold greater predicted mass transfer of these compounds to surface waters in those cities, particularly those with higher proportions of built-up areas. This is expected to increase the exposure of associated aquatic ecosystems surrounding wetter, more urbanized cities and to cause accumulation in water cycles for the more soluble OPEs that are known to be poorly treated by conventional water treatment processes. Together, these large inter-city differences in the predicted emissions and fates illustrate how the climatic and geographic characteristics of a city can be drivers of large differences in aquatic and urban terrestrial ecosystem exposures.

8.04.V-19 Mercury Releases to Environment From Mercury-Containing Products

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Mercury is a silvery-gray glittery metal liquid at room temperature. It has a high thermal expansion coefficient and forms alloys with several metals, including gold and silver. These characteristics led to several important applications, such as its use in thermometers, blood pressure measuring devices, electrical switches, in gold and silver mining operations. Mercury is among the top ten dangerous chemicals, its hazardous effects pose a serious threat to human health and the environment, as entering the latter mercury undergoes various transformations. One of the main factors among dangerous effects of mercury on humans is the mercury-contaminated natural environment, components of which penetrate into the human body with atmospheric air, surface and ground- and underground waters through respiratory tract, skin, and, with food, to gastrointestinal tract. Mercury is a rare element in nature. One-third of mercury enters the environment from natural sources, while two-thirds are due to human activity. For comparison, in the earth’s crust and main types of rock, the average mercury content is 0.03-0.09 mg /kg, while one mercury lamp, depending on its design, may contain 20-560 mg mercury. Fluorescent lamps contain mercury vapors, which can cause harm to the human body upon lamp breaking down and inability to take timely measures to neutralize it. Other hazards are due to the spectrum of ultraviolet rays emitted by fluorescent lamps, which can cause inflammation of skin (dermatitis), eye mucous membranes (cataracts), and tumors. If broken in a closed area, over time the density of mercury vapor from the lamp exceeds the allowable limit. When inhaled, mercury vapors easily pass through airways to lungs, enter the brain with the bloodstream and bring forth symptoms of poisoning. From 2016 to 2020, import of fluorescent lamps to Armenia decreased 9 times, import of sodium lamps - 10 times. Mercury emissions/ releases decreased as well. Mercury emissions/releases from fluorescent lamps through different pathways estimated in kg/year in 2016-2018 were as follows: 2016: 0.0493 to air; 0.148 to water; 0.296 to waste; total of **0.4993**; 2017: 0.021 to air; 0.063 to water, 0.126 to waste; total of **0.21**; 2018: 0.0116 to air; 0.0349 to water; 0.0697 to waste; total of **0.116**. In order to prevent mercury emissions from fluorescent lamps to different matrices, their environmentally sound collection and treatment /recovery are required

8.04.V-20 Potential Risk of Bioaccumulation of Heavy Metals in Fish Tissues of the Tunal River in Durango, Mexico

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Heavy metals pollution is a major environmental problem in water resources as it threatens public health due to its toxicity and high persistence in the environment. Water pollution supplies metal transportation to other organisms by dietary or surrounding water. Also, this effect allows bioaccumulation of metals in fish tissues. Limited data of bioaccumulation of toxic elements in fish is currently available in some freshwater systems in Mexico, where the Tunal River is one of them. Importance of bioaccumulation monitoring relies in health risk of polluted fish intake, considering that this river receives wastewater effluents. For this reason, this study aims to assess the concentration of As, Cd, Pb and Zn in water and common carp (*Cyprinus carpio*) in a

fishing area of the Tunal River in Mexico. The Tunal River is located in Durango, in the northern of Mexico. This river supplies hydric resources for different activities. Also, this river receives sewage discharges. Samples of water and muscle and liver fish tissue were taken in dry (n=7) and rainy season (n=10), as seasonal variation might influence the availability of metals in the river. Heavy metals were analyzed with an Atomic Absorption Spectrophotometer. The presence of heavy metals was confirmed in all fish samples. Only arsenic was the metal detected in water. As and Zn (>1.0 ug/g w.w) are the predominant metal in both tissues. None of the elements exceeded the maximum residual levels (MRL) allowed in Mexico. However, As and Cd in fish tissues exceed the limits allowed by international agencies. Bioaccumulation Factor (BAF) in muscle and liver for arsenic during dry season was 33.6 and 161; during rainy season 87.8 and 66.5, respectively. Some factors such as length, weight, and accumulation in other tissues, could contribute to these results. Bioaccumulation rate of Cd, Pb and Zn was not evidenced. The risk to accumulate toxic elements in liver is higher compared to muscle, indicating that common carp from the Tunal River represents a strong organism accumulative of arsenic. This study remarks the importance to carry out periodical monitoring research in order to minimize toxic effects of heavy metals in fish that habits the Tunal River.

8.04.V-21 Prospective Environmental Assessment of Cathode Active Material for Cobalt-Free Cell Battery

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This study explores the environmental performance of active material for cathode for the next generation of Co-free LIB for EVs following the ISO 14044:2006 standard for LCA, which can evaluate the different stages over the lifetime of a product. Environmental metrics with their respective methods are used for the evaluation of material, involving climate change and mineral resources depletion and scarcity concerns, among others. The cathode active material is the main environmental contributor and Co-free oxides being studied currently in the European COBRA project can reduce considerably the environmental impact by 53% compared to the conventional NMC111 and NMC622 powder production, measured in the Metal Surplus Ore Potential (kg Cu eq), that reflects the depletion and material scarcity issues. These results contribute to the promotion of Co-Free lithium-ion compositions to provide new insights into the role of cobalt, and its corresponding impacts in EVs to support the renewable energy transition.

8.04.V-22 Selection of Biocidal Active Substance Relevant to an Adverse Outcome Pathway Focusing on Endocrine Disruption Effects

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Considering that the hypothalamic-pituitary-thyroid axis and the hypothalamic-pituitary-gonadal axis interact in the case of terrestrial and aquatic vertebrates, it is necessary to present a comprehensive mechanism for thyroid dysfunction and reproductive toxicity; it may help to understand the overall endocrine system. This study aims to establish an adverse outcome pathway of endocrine-disrupting effects and to select biocidal substances related to the AOP using a big data platform (ECOTOX and ToxCast). In this study, hyperandrogenism, an endocrine-disrupting effect in females, was set as the final AOP endpoint. Based on the previously constructed AOP No. 271, additional literature research was conducted, and we proposed to extend the AOP network that organically connects thyroid dysfunction and reproductive toxicity. Finally, several biocidal substances were selected relevant to AOP. As a result, 76 biocidal active substances (e.g., Deltamethrin, Cyfluthrin, Pyrethrins, Bromadiolone Bifenthrin, Etc.) were associated with AOP. It is confirmed that exposure to these biocidal active substances could cause thyroid and reproductive toxicity by reacting with androgen receptors or thyroid hormone receptors. Future studies need to expand the list of AOP-related biocidal active substances by using an artificial neural network model for substances that do not have toxic data on the predictive toxicity big data platform. Acknowledgment: This research was supported by the Korea University Graduate School Junior Fellow Research Grant.

8.04.V-23 Should and Can Ozone Exposure Be Better Assessed?

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Ground-level ozone (O₃) is a secondary air pollutant that is a major public health and environmental issue. There is a need to precisely and exactly assess its spread in the environment and the exposure of humans and organisms. Modeling tools have been developed for this purpose but regional models need to be improved. The objective of this study was to optimize a transnational modeling defined daily at the kilometric scale (9 km²) and to quantify its capability to better estimate ozone exposure. The transnational modeling platform PREV'EST, covering eastern France and the boundary areas of Germany, Switzerland, and Luxembourg, produced daily estimations of ozone levels from 2008 to 2019 on a 3 km x 3 km grid mesh. First, we compared the daily modeled ozone concentrations (PREV'EST) to the daily values measured at 6 air quality monitoring stations. The following performance indicators were used: MBE (mean bias error), MAE (mean absolute error), RMSE (root mean square error), r (Pearson correlation coefficient). Second, three optimizations were built using multivariate adaptive regression splines (MARS). Each optimization was based on different sets of environmental variables (among mean temperature, rainfall amount, wind speed, relative humidity, elevation) including the date. Performance was assessed using the same indicators as cited previously. Third, external validation was performed using 6 additional air quality monitoring stations. The raw modeling data presented a lack of precision (RMSE = 23.18 µg.m⁻³, MAE = 19.23 µg.m⁻³) and exactitude (MBE = 14.67 µg.m⁻³), and thus needed to be improved. The optimizations improved both precision (RMSE = 11.92 - 13.60 µg.m⁻³, MAE = 9.40 - 10.81 µg.m⁻³) and exactitude (MBE = 0.01 - 0.04 µg.m⁻³), and the interday variability of the ozone levels. The external validation confirmed the performance improvement using these corrections. We propose a validated methodology that improves the model's performance and better

depicts the temporal variability of O₃. This methodology is accessible, fast-processing, and presents low computational cost and better performance. The better detection of ozone peaks will be of paramount importance to assess the potential association between acute ozone exposure and human and organism health effects, particularly in the climate change context.

8.04.V-24 The Effect of a Planktivorous Fish on the Vertical Flux of Microplastics

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Microplastic pollution of both marine and freshwater environments is currently one of the most intensely studied issues in the field of ecology and environmental protection. Many studies have attempted to estimate the distribution and concentration of microplastic particles (MP) in various environments, and determine how they affect the organisms inhabiting these environments. Decidedly less research concerns the opposite dependence, i.e. how living organisms affect the distribution, concentration and qualitative characteristics of MP. We have tested the hypothesis that fish increase the vertical flux of MP to sediments, which would be the result of the combined effect of increased sedimentation rate and the active transport resulting from vertical migration of the fish in the simultaneous presence of a thermal gradient (and in turn water density gradient) with lower meta- and hypolimnetic temperature. We conducted eight 5-day-long experiments in twin 2-metre-long vertical water columns, one of which continuously held four planktivorous fish inside. Both columns had suspended polystyrene microbeads of 25 and 250 μm in size, with density of 1000 and 200 × L⁻¹, respectively. In addition, both columns held sediment traps inside at depths of 30, 60 and 150 cm. The fish inside the column were fed small portions of dry food (immediately eaten) five times per day, and their depth selection behaviour was monitored before and after feeding by cameras. At the end of experiments, we counted the MPs caught in sediment traps as well as MPs that were still suspended in the water column at depths of 0, 30, 60, 90, 120, 150 and 160 cm. We found that the presence of planktivorous fish significantly increased the number of both larger and smaller MP that settled down in sediment traps and reduced the number of MP suspended in water. This effect is most likely attributed to increased sedimentation rate of MP in the fish treatment, rather than to their active transport, since we found that MP in the fish treatment were covered to a greater extent with a detritus than in the control, and the fish resided constantly in the subsurface layer in the water column. The study was financed by Polish National Science Centre grants (2018/31/N/NZ8/03269 and 2019/35/B/NZ8/04523).

8.04.V-25 Understanding the Contribution of Coatings to Nanoparticles Toxicity in Zebrafish

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For improving their physicochemical properties, nanoparticles (NPs) are coated with chemical compounds. Titanium dioxide (TiO₂) NPs have been modified by sodium citrate or silane molecules to enhance their colloidal stability and their photocatalytic properties. Understanding the contribution of the coatings for the toxicity of NPs is still lacking. Therefore, the aim of this work was to assess the effects of TiO₂ NPs coated with either N-(Trimethoxysilylpropyl)ethylenediaminetriacetate (silane-EDTA) or sodium citrate in embryos of zebrafish “Danio rerio”. Two embryo toxicity assays were performed to evaluate the effects at the individual and molecular levels: a development assay and a Nile red staining assay. In each assay, the exposure to TiO₂ NPs with a diameter of 45 nm combined with each one of the coatings (ratios TiO₂:coating: 1:1.5 wt for citrate and 1:0.004 wt for silane-EDTA) was compared to groups exposed only to the coatings (“single exposure”) and to the negative control group (no coating). Firstly, in the development assay, zebrafish embryos were exposed to TiO₂ NPs (5, 50 and 200 mg/L) with one of the two coatings, from 0 to 96 hours of post-fertilization (hpf), following OECD guideline nr. 236. Every 24 hours the mortality, hatching, development and malformations were checked and the length and the heartbeat were measured at the end (96hpf). To study the expression of target genes by qPCR (i.e. genes related to oxidative stress, lipid and energetic metabolism) the larvae were stored in RNA later at -80 °C. Secondly, the effect of TiO₂ NPs in lipidic biomolecules was analysed from 48 to 120 hpf with the Nile Red assay (25 and 500 mg/L). The results of the Nile Red assay allowed us to quantify effective regional adiposity by fluorescence stereomicroscopy. The results of this work will allow us to understand whether different coatings have additive, synergistic or antagonistic effects comparing to the toxicity of TiO₂ NPs in zebrafish.

8.04 Extended submission WED (Poster)

8.04.P-We291 A Non-Target Screening-Directed Long-Term Time-Trend Study of Organic Contaminants in the Baltic Top Consumers

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The key species of the Baltic ecosystem suffer from impaired health. It is believed that anthropogenic hazardous substances along with some halogenated natural products are one of the reasons for that, as they accumulate in organisms. Since international restrictions on hazardous chemicals went into force in the 1970-80s, time trends of many environmental persistent organic pollutants (POPs), including PCBs, DDT and its metabolites, and others, have been decreasing constantly for decades. However, the amounts of legacy POPs in the Baltic Sea region have leveled off and stopped declining recently and new contaminants are frequently discovered. There is, hence, a need for comprehensive approaches to study the occurrence and time trends of the legacy POPs and contaminants of emerging concern (CECs), amounts of which are increasing, in the ecosystem. Here, we report the first

time-trend study which utilizes a non-target screening (NTS) approach for this purpose. The studied samples included common guillemot egg extract (1986-2019, 12 samples), white-tailed sea eagle muscle (1965-2017, 8 samples), and harbor porpoise blubber (1988-2019, 9 samples). A major challenge in NTS of biota is the removal of matrix components, like lipids that may interfere with the detection and identification of analytes. A sufficient level of lipid removal was achieved by a combination of high-resolution gel permeation chromatography and Florisil fractionation. The data were acquired using GC-QTOF/MS in both electron ionization (EI) and electron capture negative ionization (ECNI) mode to maximize contaminant coverage. Acquired EI data sets were processed using a new highly-automated adjustable workflow. The ECNI data were manually processed and reviewed. The Mann-Kendall test was used to discover time trends and the Theil-Sen regression was used to estimate annual change for the features exhibiting significant time trends. Altogether, more than 300 tentatively identified contaminants were found to have significant time trends in samples studied. Significant decreases were found for many regulated chemicals, as could be expected, e.g., PCBs, DDT, and other organochlorine pesticides. Significant increases were observed for, e.g., small PAHs, heptaBDEs, and CECs. The CECs include, among others, a novel plasticizer tributyl acetylcitrate and two compounds used in polymer production trimethyl isocyanurate and 2-mercaptobenzothiazole, which have not previously been reported in biota.

8.04.P-We292 Analytical Method to Measure Gases That Contribute to Climate Change and Impact COP26 Glasgow Climate Pact

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Fossil fuel consumption increases the concentration of greenhouse gases (GHGs)— such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O)—in the Earth's atmosphere. These gases trap heat, thereby affecting our planet's temperature. Recently, at the Glasgow Climate Change Conference (COP26) nations came together to make decisions and limit global temperature rise to 1.5 C. GHG's are a large contributor of climate change and temperature increase. To help mitigate climate change caused by increased concentrations of atmospheric GHGs, regulatory institutions (such as the EPA and CEN) have initiated programs to inventory GHG emissions through continuous measurement which are even more important now. GHG analysis requires GC detectors specific for each of the targeted gases. Methane is analyzed by Flame Ionization Detectors (FID), carbon dioxide by thermal conductivity (TCD)m and nitrous oxide by electron capture (ECD). In addition to these detectors, sample introduction is an important consideration. Detection ranges for these compounds often ranges from ppb on the trace side and extending well into percent levels. Historically, GHG samples were introduced to a GC system by gas sampling valve utilizing either a pressurized sample container or a gas tight syringe. Increased interest in GHG levels around the globe have created a need for more capacity and higher levels of automation, in addition to faster run times. A custom GC option for GHG analysis is evaluated here in this study as the 8890 Greenhouse Gas Analyzer. Detection capabilities, improvement in run time, and sampler considerations are discussed to demonstrate compliance with current EPA and CEN methodologies and regulations. A flexible, yet fast and robust GC configuration for measuring CH₄, N₂O and CO₂ from ppb to % level on three dedicated detectors in less than 5 minutes was developed and method performance criteria will be shown as well.

8.04.P-We293 Antibiotic REsistanNce and Pathogenic Signature in Marine and Freshwater Aquaculture Systems. ARENA Project

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An increasing number of studies have reported the widespread occurrence of antibiotic residues (ABs) in freshwater and marine environments, raising concern on potential ecological and human health risks. Aquaculture represents a key source of fish and other foods for human consumption and a suspected hotspot for AB release and product contamination. In this scenario, the ARENA project aims at improving the quality assessment of aquaculture practices and products by exploring sources, reservoirs and pathways of antibiotic and microbial contamination across the water cycle. Contamination levels will be addressed from sources to end points and final food products (e.g., cultured fish fillets), within recirculating (i.e., RAS) and open aquaculture systems (i.e., mariculture), as well as in the surrounding marine environment. Sampling at two seasons with contrasting anthropogenic pressure will be performed to account for possible seasonal variations. Specifically for AB analysis (WP3), a multi-residue LC-MS/MS method will be implemented to monitor the presence and concentration of selected ABs and their metabolites in different environmental matrices from both RAS systems (i.e., water, farmed fish, feed) and mariculture in the Adriatic Sea (i.e., water, farmed fish, feed, sediment, and benthic meio- and macroinvertebrates below farm cages). A suspect screening strategy based on High-Resolution Mass Spectrometry (HRMS) will also be applied to identify additional ABs, metabolites, and transformation products. In addition, a novel flow-cytometric tool will be tested for the rapid detection of AB residues in edible fish products, and its performance compared against the targeted LC-MS/MS method. Alongside with the screening of ABs, early-warning tools will be optimized for the rapid detection and quantification of antibiotic-resistance genes (high-throughput sequencing-based) and microbial pathogens (impedance spectroscopy-based) in environmental and fish food samples. Altogether, we expect that the ARENA project will provide a cross-disciplinary approach for AB risk assessment and contribute to mitigate AB resistance and pathogenicity in aquaculture settings.

8.04.P-We294 Are Accumulated PFAS Concentrations in Benthic Macroinvertebrates Related to the Ecological Quality of Aquatic Environments?

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Benthic macroinvertebrates play a key role in freshwater ecosystems by driving ecosystem services such as nutrient cycling, sediment mixing, and energy flow through the food web. Because some taxa are more sensitive to pollution than others, the macroinvertebrate community structure can show the cumulative impact of short- and long-term pollution events. However, monitoring micropollutants in aquatic ecosystems is still mainly based on measurements in the abiotic environment. Sediment and water samples represent only a momentary pollution status and do not take into account differences in bioavailability driven by fluctuating abiotic and biotic factors. Consequently, derived water and sediment quality criteria might not always be adequate for the protection of aquatic communities. Despite the increasing awareness of the widespread usage and ubiquitous presence of Perfluorinated alkyl substances (PFAS) in the environment, little is known about their effects on macroinvertebrate communities. The aim of this research is to study the relationship between accumulated PFAS levels in three benthic invertebrate species (*Chironomus* sp., *Asellus* sp., and *Gammarus* sp.) and the aquatic ecological status. Therefore, resident invertebrate taxa are collected at 28 sites in rivers and watercourses across Flanders, Belgium. To assess the invertebrate community responses, the ecological water quality index, also known as the Multimetric Macroinvertebrate Index Flanders (MMIF), is calculated. Simultaneously, sediment and water samples are collected to investigate possible relationships between accumulated PFAS in the biotic and abiotic environment. In addition, we aim to identify threshold body burdens of PFAS in order to protect aquatic communities.

8.04.P-We295 Assessing the Risk to Freshwater Ecosystems From Water-Soluble Polymers

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Over the last decade, concerns over water-insoluble polymers such as micro and nanoplastics have dominated the field of environmental plastic pollution, but until recently, water-soluble polymers (WSPs) have been largely excluded from the conversation. WSPs are a wider umbrella for a range of polymer classes differing in their chemical behaviour as well as their direct and indirect toxicities to freshwater organisms. Their use in personal care products (PCPs), industry, agriculture and more is expected to increase, particularly with new legislation reducing the use of micro and nanoplastics. Commonly used WSPs such as polyacrylamide are expected to see a market growth of 6.2% from 2019-2025 which supports an output of around 2.5 million tons globally per year. Therefore, due to their high production volumes, vast applications and solubility their discharge into the environment is inevitable. One of the major routes of environmental exposure is likely to be through wastewater systems, with evidence of WSPs not being fully removed in the treatment process, along with the increasing issue of raw sewage discharge into freshwater. As well as this route, usage in fertiliser and pesticide formulations and widespread application as a soil conditioner could also see run-off into nearby water bodies. In addition to point sources, non-point source (NPS) applications in cements and paints could also contribute to further leaching. Despite their expected prevalence in the environment, research into WSPs is hindered by the lack of prior research and availability of analytical methods. Size-exclusion chromatography methods such as gel-permeation chromatography (GPC) require the concentration of the polymer to be known to calculate an accurate molecular weight, while common mass spectrometry used in the analysis of polymers, such as matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF), require precise sample preparation to detect a range of WSP classes. Here, we propose a novel sample extraction and separation utilising both GPC and MALDI-TOF to identify WSPs. Alongside analytical methodology, the development of a mass spectral PCP database can be used to assist in the identification of WSP molecular fingerprints. Utilising a combination of novel extraction methods and market products is aiding the identification of WSPs present in wastewater and could be used on environmental samples to identify polymers of concern in freshwater ecosystems.

8.04.P-We296 Boreal Lake Ecosystem Responses to a Chronic Venlafaxine Exposure

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Antidepressant drugs are present in freshwater ecosystems worldwide. Venlafaxine, a serotonin norepinephrine reuptake inhibitor, has been detected in freshwater at >2.0 µg/L. Its effects have been well studied in laboratory settings and it is known to alter neurological genetic expression and behavioural responses in fish. However, its possible effects have not been investigated in a whole ecosystem context where indirect effects of chronic exposure could pose a risk to long-term ecosystem health. Here we show the response of primary producers and consumers after a 10-week exposure to venlafaxine. Sediment-inclusive mesocosms were deployed in-lake at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA), a boreal lake research facility in northwestern Ontario, Canada. Mesocosms (n=10, 2m diameter, 1.5m deep) contained a native assortment of plankton and invertebrates and were each stocked with five finescale dace (*Chrosomus neogaeus*). After acclimating, mesocosms were spiked weekly with venlafaxine at seven nominal concentrations ranging from 0.004 µg/L to 100 µg/L (n=1 per treatment), plus 0 µg/L (control; in triplicate). Simultaneously, in-lab, standard 7-day embryo-larval assays were conducted using wild collected fathead minnow eggs exposed to mesocosm water, which were used for behaviour response testing. Treatment effects assessed were the biomass of periphyton, abundance of benthic and emergent macroinvertebrates, behaviour of larval fathead minnows, and condition of finescale dace after 10 weeks of exposure to venlafaxine. For most endpoints minimal responses were observed at environmentally relevant concentrations, although behavioural and water quality impacts were observed in our higher treatments. Our study provides higher tier effects data on the integrated responses of a whole ecosystem to the nuanced and sublethal effects of one of the most commonly detected psychoactive contaminants.

8.04.P-We297 Ecotoxicological Assessment of Surface WATER Samples Contaminated by Tailings From Iron Ore Dam Rupture

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The rupture of tailing dams causes numerous environmental impacts to the watershed in the affected region. As tailings reach surface water, water turbidity, suspended solids, dissolved solids and biological oxygen demand increase instantaneously, thus impacting aquatic life. Increased levels of iron have also been associated to the rupture of iron mining dams in Brazil. These effects may be seen hundreds of kilometers from the former dam as tailings are transported by river water. This work aimed at the analysis of acute and chronic toxicity of surface water impacted by the rupture of an iron ore dam in the Southeastern region of Brazil (currently under secrecy). Microtox® acute toxicity test and chronic toxicity test using *Raphidocellis subcaptata* (72h) were performed as according to national standards (ABNT NBR 15411-3:2021 and ABNT 12648:2018) for twelve surface water sampling points (3 reference points and 9 sampling points in the affected region) in the dry (September) and wet (December) seasons. Samples were taken months after the rupture event. No acute toxicity was observed for any of the sampling points as expected due to long interval between the dam rupture and sampling since tailing settle in the river sediments. However, chronic toxicity to green algae was detected for 6 sampling points in the dry season (2 reference points, 2 inside the former dam area and 2 downstream the former dam) and two sampling points in the rainy season (one reference point and one inside the former dam area). Chronic effect to green algae had been detected with samples taken days in the same watershed after the rupture event and this organism was the most sensitive to the effect of tailings components when compared to crustaceans and fish. Dilution of samples during the rainy season may have affected chronic toxicity as less points were toxic. Despite the detected effect, chronic toxicity may not be directly associated to the rupture as chronic toxicity was also detected in reference points probably due to the influence of other activities performed in the watershed (agriculture, sewage disposal, industrial wastewater disposal). These results reveal the need for continuous monitoring of toxicity in this watershed along the years considering the long-term effect of mining and other human activities performed in the watershed.

8.04.P-We298 Effects of Cadmium and Copper, Isolated and Combined, to a Freshwater Neotropical Copepod - a Short-Term Exposure

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Metal contamination is of great concern in aquatic ecosystems and can result from anthropogenic and natural processes. These contaminants interfere in biological processes, affecting the whole trophic chain. We evaluated the impact of cadmium (Cd) and copper (Cu), isolated and combined, to a Neotropical freshwater Calanoida Copepoda (*Notodiaptomus iheringi*), assessing the responses of lethality, dry weight, and lipid classes. The animals were exposed for 48 h to control, five concentrations of each metal and six mixtures (Cu+Cd). The lethality results were evaluated using the MixTox tool, and two-way ANOVA was used to assess the lipid and dry weight responses, with $p < 0.05$ and Fisher LSD test. One concentration of each metal and its combination were assessed for the lipid class composition. The LC50 of Cu and Cd was 27.7 and 8.0 $\mu\text{g L}^{-1}$, respectively. The metals combination resulted in antagonistic effects in the lethality to the copepod, with the independent action and S/A fitting better the results. The contaminants decreased the mean dry weight of the organisms and presented significant differences from the control ($p < 0.05$). The organisms from control presented phospholipids (PL; $\approx 50\%$), triacylglycerols (TAG; $\approx 30\%$), and acetone mobile polar lipids (AMPL; $\approx 11\%$) as the main lipid classes. Compared to control, Cd decreased the TAG ($\approx 20\%$; $p < 0.05$) and increased the sterols (ST; $\approx 6\%$; $p < 0.05$) without affecting PL ($\approx 51\%$; $p > 0.05$) or AMPL ($\approx 11.5\%$; $p > 0.05$). Cu significantly decreased PL ($\approx 31\%$; $p < 0.05$) and AMPL ($\approx 7\%$; $p < 0.05$) and increased TAG ($\approx 51\%$; $p < 0.05$) and free fatty acids (FFA; $\approx 7\%$, $p < 0.05$). The combined metals did not affect significantly PL ($\approx 42\%$; $p > 0.05$), decreased AMPL ($\approx 7\%$; $p < 0.05$) and increased TAG ($\approx 48\%$; $p < 0.05$). The change in lipid classes in Cd treatment can indicate an alteration in membrane conformation (increasing ST) to reduce metal internalization. In contrast, the changes in Cu and mixture treatments indicate a redirection to reserve lipids (increasing TAG) instead of structural lipids (decreasing PL) to deal with the stress. Our results suggest that Cu and Cd, isolated or combined, affected survival, with antagonism and independent action observed; besides affecting the dry weight and lipid composition of *N. iheringi* in 48 h. Ecotoxicology studies using copepods are scarce. As far as we know, this is the first study using this species; thus, we suggest more studies to evaluate this species as a new test organism in Brazil.

8.04.P-We299 Evaluating the Use of Cattail (*Typha latifolia*) Wetlands for Land-Based Aquaculture Wastewater Effluent Remediation

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An increasing demand for fish and fishery products has been met by a steady increase in aquaculture production, making it one of the fastest growing food production sectors in the world. Land-based aquaculture systems continue to rise in popularity as they offer the benefit of controlling diseases, managing water quality, and minimizing threats to wild populations. However, aquaculture systems generate wastewater high in nitrogen and phosphorous. As with any agricultural output, the nutrient-rich effluent generated in aquaculture systems have the potential to lead to eutrophic conditions in receiving waters and threaten the health of aquatic ecosystems. Consequently, a number of conventional wastewater treatment methods have been applied to aquaculture systems. Constructed wetlands are a promising and cost-effective strategy for treating wastewaters, and offer the

additional benefit of recycling nutrients that would otherwise be wasted. Here we show how cattail (*Thypha latifolia*), an abundant and resilient wetland plant, can be used to remediate wastewater effluent from land-based aquaculture systems. Model cattail wetlands were established in mesocosms (2 m diameter) and had a gradient of aquaculture wastewater applied weekly, for a total of five weeks. Five wetlands received wastewater (in low to high concentration) and three received no wastewater (i.e. reference treatments). Model wetlands were robust to changes in water quality; a $\sim 0.15 \text{ mg L}^{-1}$ increase in each of total phosphorous, total dissolved phosphorus, and soluble reactive phosphorus was detected for wetlands dosed the largest volumes of wastewater. As wetlands proliferated over time, these concentrations returned to background levels ($< 0.05 \text{ mg L}^{-1}$). Mean plant biomass was found to be $\sim 70\%$ greater in the high treatment relative to the reference treatment. Nutrient analysis of plant material revealed increases in total Kjeldahl nitrogen, total nitrogen, and total phosphorous among high treatments. No trends were observed among other nutrients analyzed (i.e. total sulfur, nitrate, B, Ca, Cu, Fe, K, Mg, Mn, Zn). Our results suggest that cattail wetlands are a viable option for treating effluent from land-based aquaculture operations. Additionally, the implementation of this remediation strategy shows potential for nutrients to be recycled in cattail plants, which can then be harvested for use in subsequent applications (e.g. as biofuel, as fertilizer following composting).

8.04.P-We300 Exploration of Abundance, Distribution and Composition of Microplastics ($>10\mu\text{m}$) in Marine WATER *Yuanli Liu¹, Claudia Lorenz¹, Alvise Vianello¹, Kristian Syberg², Asbjørn Nielsen¹, Torkel Gissel Nielsen³ and Jes Vollertsen¹, (1)Aalborg University, Denmark, (2)Roskilde University, Denmark, (3)DTU Aqua, Denmark*

The sea between Denmark and Sweden, the Kattegat, was mapped for microplastics (MPs) in its upper water layers. Samples were taken by pump filtration along a continuous transect of 900 km, filtering a total volume of 19.344 m^3 on $10 \mu\text{m}$ steel filters. The transect was divided into 13 individual samples and analyzed down to $11 \mu\text{m}$ in MP size applying μFTIR hyperspectral imaging. MP concentrations spanned from 18.93 to 289.78 MP/m^3 with an estimated mass concentration of 0.6 to $84.1 \text{ } \mu\text{g/m}^3$. Concentrations tended to be high in and ‘downstream’ of the Øresund region, which has a population of nearly 4 million, indicating an MP footprint on the coastal waters. There was however no correlation between closeness to urban sources and particle size distribution nor polymer composition. Most of the identified MPs (57%) were below $100 \mu\text{m}$ in their largest dimension and the most common polymer was Polyester, followed by Polypropylene and Polyethylene. The correlation between concentrations measured as MP number and MP mass was weak, indicating that calculating one based on the other should be done with care.

8.04.P-We301 Exploring Stakeholders' Attitudes and Use of Swedish Knowledge Support Databases for Pharmaceuticals and Environment: A Qualitative Study

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Introduction: Pharmaceuticals are vital for human and animal health, but can pose a threat to the environment. Moving towards a reduced pollution society requires mitigation strategies to reduce the risk of pharmaceuticals in the environment. In Sweden, two publicly available databases exist that offer knowledge support and information on the effect of pharmaceuticals in the environment. They are referred to as “Pharmaceuticals and Environment” (www.Janusinfo.se), and “Environmental Information” (www.Fass.se). The objective of this study was to explore stakeholders' attitudes, and use of these two databases, as well as discuss related initiatives to mitigate the negative effects from pharmaceutical residues in the environment. **Methods:** Two interviewers conducted semi-structured interviews with 21 stakeholders regarding the Swedish knowledge support databases for pharmaceuticals and environment. Interviews were conducted from November 2021 – December 2021. Stakeholders represented the pharmaceutical industry, regional, and national authorities. The interviews encompassed the development, use, strengths and limitations regarding the databases as well as other initiatives. The two interviewers independently conducted thematic analysis on the first interviews to derive the themes, which were validated by the last author. The first author completed the remaining analysis using NVIVO®, a qualitative analysis software. **Results:** Major themes that emerged from the interviews included 1) challenges and opportunities faced with using the environmental information on the knowledge support databases, 2) transparency, 3) user-friendliness, and 4) other initiatives related to the knowledge support databases. **Conclusions:** In general, stakeholders appreciated the availability of the databases. Stakeholders stated that they mainly used the databases to decide which pharmaceutical to recommend in their treatment guidelines when two pharmaceuticals had equal efficacy and safety profiles, and for research. Stakeholders preferred using the environmental information on *Janusinfo* for treatment guidelines because of how the information was presented, and due to credibility. Stakeholders spoke about other initiatives relating to transparent manufacturing practices, and fixing the data gap. With the experiences from this study, opportunity exists for collaboration on international knowledge support databases to reduce the risks from pharmaceutical residues in the environment.

8.04.P-We302 Fate of Single Use Plastics (SUP) Exposed to Weather, Placed in Seawater and Buried in Sediment

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Single-use plastics (SUP) comprise the most frequent materials in plastic litter collected in more than 700 European surveys of terrestrial, beach and marine environments since 2016. As a result, the EU's Directive on single-use plastics was implemented in July 2021 to restrict their availability and reduce their impact on the natural environment. Despite the focus on SUP, there is a significant lack of data describing degradation pathways and -rates of SUP on land, underwater and in marine sediments. In this research, we establish for the first time the primary drivers and timescales of fragmentation for new SUPs in the form of cups, coffee lids, bottles, containers and cable ties with similar thicknesses, dimensions and profiles. Our SUP set comprised

polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET) and polyamide-66 (PA) in transparent, white and black. As part of research project, Velux MarinePlastic, the SUP were mounted on frames and exposed to Danish weather, underwater (ca. 1m) and buried in sediment (75cm) in Lynæs Harbour. Accurate temperature, ultraviolet and visible light measurements were recorded at 12-hour intervals. Plastics were also exposed to accelerated thermal ageing at $70\pm 2^\circ\text{C}$ to estimate the period for new SUP to fragment to microplastics, smaller than 5mm. Changes in dimensions and appearance were examined at 6-month intervals using optical microscopy, changes in chemical structure were determined using Attenuated Total Reflection Fourier Transform infrared spectroscopy and the loss of additives and formation of degradation products detected using Gas Chromatography-Mass Spectrometry. After 24 months, results indicate that both environment and plastic formulation are important factors in the degradation of SUPs. Degradation was fastest at 70°C , followed by exposure to Danish weather, underwater and sediment. Chemical markers suggested oxidation to be the primary degradation pathway for all SUPs. Plastics exposed to Danish weather degraded faster than those underwater or in sediment due to the difference in light levels. Black SUPs degraded significantly slower than their white or transparent equivalents. SUPs composed of PE, PS and PP developed measurable chemical and physical degradation markers in all environments, while PET and PA showed greater resistance to oxidation. Exposure of SUP to thermal ageing, Danish weather, underwater and sediment continues.

8.04.P-We303 Food Contact Materials: An Effect-Based Evaluation of the Presence of Hazardous Chemicals in Paper and Cardboard Packaging

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Food contact materials can contain hazardous chemicals that may have the potential to migrate into food and pose a health hazard for humans. Previous studies have mainly focused on plastic materials, while data on packaging materials made from paper and cardboard are limited. We used a panel of cell-based bioassays to investigate the presence and impact of bioactive chemicals on human relevant endpoints like oxidative stress, genotoxicity, inflammation, xenobiotic metabolism and endocrine system effects in extracts made from paper and cardboard. In total, twenty-three methanol extracts of commonly used paper and cardboard available on the Swedish market were extracted as a whole product using methanol to retrieve polar substances, and tested at concentrations 0.2 – 10 mg/mL. At the highest concentration bioactivities were observed in a high proportion of the samples: oxidative stress (52%), genotoxicity (100%), xenobiotic metabolism (74%), antiandrogenic- (52%) and antiestrogenic receptor (39%). Packages of potential concern included cake/pastry boxes/mats, boxes for infant formula/skimmed milk, pizza boxes, pizza slice trays and bag of cookies. It should be noted that the extraction for packages like cake/pastry boxes can be considered exaggerated, as the exposure usually is shorter. It can be hypothesized that the observed responses may be explained by inks, coatings, contaminants and/or naturally occurring compounds within the material. To summarize, an effect-based approach enables hazard identification of chemicals within food contact materials, which is a valuable tool for ensuring safe use of these types of package materials.

8.04.P-We304 Investigation and Analysis of Microplastics in Sewage Sludge and Biosolids: A Case Study From One Wastewater Treatment Works in the UK

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There is an increasing concern about the impacts of microplastic pollution in the terrestrial environment. The recycling of biosolids to land can create a pathway for microplastic contamination of agricultural soils and in the UK alone, 3.5 million tonnes (wet weight) of biosolids from the wastewater industry are recycled each year to agricultural land. This raises the possibility that recycling of biosolids could be a significant source of microplastic pollution to the terrestrial environment. To address this issue, the present study determined the occurrence and characteristics of microplastics from across the whole sludge treatment stream from one exemplar wastewater treatment works in the UK and assessed what this may mean in terms of soil contamination. Both sewage sludge (a liquid by-product produced from the wastewater treatment processes) and biosolids (sewage sludge that has undergone a treatment process) were examined as a source of microplastics to the terrestrial environment. A catalytic wet peroxide oxidation process (30% H_2O_2 and 0.05 M FeSO_4) was performed on each of the dried samples followed by density separation (1.5 g cm^{-3} ZnCl_2). Microplastics 5000 μm and 50 μm were quantified and characterised and a subsample was subjected to chemical analysis via μFTIR spectroscopy for microplastic confirmation and polymer identification. Microplastics were detected in all samples taken from across the treatment process with concentrations ranging from 37.7–286.5 MPs/g sludge (dry weight). The microplastic load in the final biosolid products produced at the site ranged from 37.7–97.2 MPs/g of sludge (dry weight). The wastewater treatment works in this study produces 900 tonnes of anaerobically digested sludge cake and 690 tonnes of lime stabilised cake per month. Based on the results from this study, the application of these biosolids to agricultural land as fertilisers can potentially release 16.1 billion and 10.2 billion microplastics in anaerobically digested and lime stabilised sludge respectively, every month (equivalent to the same volume as >20,000 plastic bank cards). The results illustrate the extent to which microplastics may enter the terrestrial environment through this route. The work carried out in this study was a preliminary investigation and paves the way for further research into the presence of microplastics in the whole sludge treatment stream.

8.04.P-We306 Localization and Impact of Perfluorooctanoic Acid (PFOA) In Vitro and In Vivo by High-Resolution Chemical Imaging

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Perfluoroalkylated substances (PFASs), such as perfluorooctanoic acid (PFOA), have been extensively used for many industrial

applications and are therefore ubiquitous in our surroundings. However, these chemicals are toxic and persistent. They accumulate in living organisms including humans, leading to major health problems. Upon oral exposure, the first physical barrier against these pollutants is the gastrointestinal tract. Unexpectedly, little is known about their impact on the intestinal wall. This study endeavors to discover the fate, uptake and impact of PFOA using *in vitro* and *in vivo* assays combined with new imaging methodology. Besides common light/electron microscopy, we use Secondary Ion Mass Spectrometry (SIMS) which has both an excellent spatial resolution and an excellent detection limit, allowing sub-cellular localization of low-level toxicants. For the *in vitro* assay, an intestinal cell line (Caco-2) was grown on inserts and acutely (24h) exposed to PFOA (0-100 μ M). Next to various toxicity assays, cells were chemically fixed and stained for high-resolution elemental SIMS imaging and electron microscopy. The samples were analyzed using a Helium Ion Microscope combined with a SIMS (HIM-SIMS, sub-20nm lateral resolution) and a Scanning Electron Microscope (SEM). For the *in vivo* assay (first approved by an ethical committee), adult male mice were acutely exposed to PFOA via oral gavage (0-100 mg/kg body weight/day for 3 days). Next to weekly follow-ups and key biomarkers analyses, the intestines (jejunum and colon) and the liver were cryo-fixed for molecular SIMS imaging. Following optical observations, the tissues were analyzed using a Time-of-Flight SIMS (TOF-SIMS, 1 μ m lateral resolution). We effectively localized PFOA inside tissues and cells. At the tissue level, PFOA is homogeneously distributed in the liver while it is localized in some specific areas in the jejunum and in the colon. In addition, we observed colocalization of PFOA with some fatty acids, probably due to their structural similarities. At the cellular level, PFOA is only localized in the cytosol of intestinal cells, but not in cytosolic lipid droplets. The combination of mass spectrometry images with usual electron/light images provides insightful information about the fate, uptake and impact of PFASs in the gastrointestinal tract and the liver and opens new opportunities in toxicology and pharmacology.

8.04.P-We307 Mercury Levels in the Wabigoon River System

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Mercury (Hg) is a toxic trace metal that bioaccumulates in the food chain. Due to its toxicity, it has been subject to several international treaties and remediation actions. The Wabigoon River is known for an historic mercury pollution source, caused by a chlor-alkali facility operating in the 60's. As legacy Hg contamination continues to cause serious adverse health effects to the local communities living in the Wabigoon River region, further research is crucial to understand the deposition and transport of the historical Hg in this system. To answer these questions sediment cores and water samples were collected at different locations along the river, including different ecosystems such as lakes, wetlands, and riverbed/river. Current Hg levels in water allows to identify critical locations of mercury hot spots, which could be sources for transport within the system. Besides that, concentrations of total Hg and methylmercury (MMHg) were quantified in sediment cores. The analysis of cores in different locations spread around the Wabigoon River System allows to also examine the temporal variation of THg and MMHg concentrations within the ecosystem. This analysis permits to establish baseline as well as current Hg and MMHg concentrations for sediments. Results from this project are important to effectively monitor the pollution spread.

8.04.P-We308 Microplastics in the Pelagic Environment: Their Presence in Lanternfish (Myctophidae) From the Western Mediterranean and Potential Transference up in the Food Web

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Microplastics are already ubiquitous in the marine environment, although their distribution along the water column and their circulation in the marine food webs is yet to be fully understood. Here, we analyze microplastic content in the digestive tract of three pelagic lanternfish species, an important group of fish in terms of biomass that occupies an intermediate position in the marine food web. We also explore their role as a vector of microplastics for one of their predators, the striped dolphin (*Stenella coeruleoalba*). Almost half of the analyzed lanternfish contained microplastics, mostly blue and black fibers (40.9% and 34.66%, respectively). The median of microplastics found per fish, in fishes with at least one microplastic, was 3 (CI 95% = 3.46 – 6.8); similar to other studies performed in other fish species in the area. Biometric parameters of fish, such as total length and body condition, were not correlated with number of microplastics. However, microplastics' frequency of occurrence in our samples (40.21%) were significantly different from the occurrence in the digestive tracts of striped dolphins from the same area (90.5%). The amount of microplastics per individual among these species was also significantly different, with a median of 1.907 items/fish (CI 95% = 3.46 – 6.8) and a median of 7 items/dolphin (CI 95% = 3 - 13), suggesting the possible biomagnification role of lanternfish in the food web in the study area. Data presented here contributes to quantify the severity of microplastic pollution in the understudied pelagic environment and in wild, non-commercial and protected species. It will also help to build modelling scenarios together with studies that target microplastic retention and egestion rate in these species.

8.04.P-We309 Microplastics Pollution in Oyster Bed Ecosystems: An Assessment of the Northern Shores of the United Arab Emirates

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Microplastics are a subgroup of the smaller plastics and have emerged as a pollutant of concern. This study assesses microplastics pollution in the largest oyster bed ecosystem in the Northern Emirates, a region of the United Arab Emirates (U.A.E). Oyster beds are important ecological, cultural, and economical ecosystems in temperate regions. The accumulation of microplastics in sediments can influence the surrounding ecosystem, therefore affecting the surrounding oyster beds. As oysters are one of the many sources of food consumption, monitoring the abundance of microplastics is important as they can pose a potential health threat to humans. Microplastics in both the sediments and oysters, were assessed for abundance, shape, size, color, and composition. Sediment samples were prepared and digested using KOH, followed by a high- density gradient separation. Oysters

samples were treated on a similar manner, with the aid of hydrogen peroxide to ensure a stronger digestion. Subsequently, samples were filtered under vacuum filtration and analyzed under the microscope. Overall, the mean abundance in the sediment samples was 191.7 +/- 95.5 particles/ Kg of d.w.; while the mean abundance in the oyster samples was 101.2 +/- 93.8 particles/ Kg of sample. No correlation was observed between the abundance of oyster and sediment samples. Fibers accounted for 93% of microplastics found in both oysters and sediments in all sites. Additionally, black microplastics were the most abundant, accounting for 53% in both the sediment and oyster samples. The most dominant size range was 1.0-2.0mm, accounting for 34% in both sediment and oyster samples. Samples were further identified using FTIR analysis, to identify the potential sources of microplastics. This is the first study to investigate the presence of microplastics in oyster beds and it provides baseline information for monitoring the microplastics pollution in coastal ecosystems of the region.

8.04.P-We310 Mycotoxins From Corn Silage Suspected As a Cause of Yellow-Headed Blackbirds Mortality in the City of Durango, Mexico

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An unestimated amount of yellow-headed blackbirds (*Xanthocephalus xanthocephalus*) died in the urban green areas of Durango city between January and March of 2021. The birds were observed debilitated, lethargic, blindness, lack of awareness of environments, inability to fly, tremors, and flapping wings. Birds were often simply found dead. The aim of this work was to determine the possible causes of the mortality of these birds. So we collected dead birds and carried out diagnostic tests that included necropsy, cultures for the determination of bacteria and fungi, RT-PCR analysis for viruses (Newcastle and Influenza type A), analysis of gas chromatography coupled to mass spectrometry (GC/MS) for the detection of organochlorine and organophosphate pesticides. The activity of acetylcholinesterase (AChE) and glutathione-S-transferase (GST) was also measured as biomarkers of exposure to contaminants in different tissues of dead birds and in apparently healthy live birds for comparison. The most relevant results were that all specimens were in good physical condition with large layers of subcutaneous fat. Analysis of esophageal and proventricular contents which consisted entirely of corn grains, no other unusual findings were seen at gross necropsy. Bacterial cultures of the birds revealed *E. Coli*, *Enterococcus* spp. and *Staphylococcus* spp. Fungi cultures of esophageal and proventricular contents revealed the presence of filamentous fungi with morphological characteristics compatible with *Aspergillus* spp. Analysis for viruses and pesticides resulted negative. Brain AChE in birds suspected of poisoning was lower than in apparently healthy birds. GST activity was higher in suspect birds. Aspergillosis in wild birds is associated with feeding birds unharvested grains and silage because *Aspergillus* fungi live on dead or decaying organic matter. Around the city of Durango, there are some farms where it is common to see the blackbirds consuming corn silage. Aflatoxins are a metabolic product of *Aspergillus flavus* and *A. parasiticus*, their distribution could not be uniform in a given sample of corn silage, some portions could be more toxic than others. This means that some birds which ingested corn silage may be found healthy birds showing no signs of intoxications, but others who ingested aflatoxins to develop subclinical or acute intoxication and death. Report of aflatoxicosis in blackbirds are not present in the literature. However, we can speculate that this species is highly vulnerable to this toxin, unlike other species of birds and mammals.

8.04.P-We311 Natural Organic Matter (NOM) and Ionic Composition Dictate Mercury (Hg(II)) Removal From Contaminated WATER by a Wood-Based Biochar

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Despite the Minamata Convention, generation of Hg(II) laden waters from anthropogenic activities continue to pose a risk in many countries. To effectively treat these waters, novel sorbent materials are being developed. But, adopting these materials on a large scale can be economically challenging. Biochar, as a cost-friendly sorbent, can be used to remove Hg(II). Multiple removal mechanisms for aqueous Hg(II) using biochar have been reported. However, removal in “clean” systems comprising of Hg(II) as ionic Hg²⁺ cannot be directly extrapolated to complex systems, where Hg(II) co-exists with NOM and/or Cl. Also, NOM can block biochar pores, and background cations can screen the negative surface charge over biochar and increase the hydrophobicity of NOM. The interrelated effects of NOM and ionic composition on Hg(II) removal, which are highly relevant in complex systems remain unclear. In this study, we investigated Hg(II) removal via a wood-based biochar beyond “clean” systems towards complex aqueous matrices. Laboratory batch experiments combined Pahokee Peat extract as a NOM representative, with major ions (Cl, NO₃, Ca, and Na) and the interaction was examined using EXAFS, 2D-μ-XRF and SEM-EDS analysis. In the absence of NOM, Hg(II) removal was driven by access to sorption sites containing S within the porous biochar. Ca enhanced removal through cation bridging between the negatively charged biochar surface and Hg(II)-chloro complexes. NOM decreased Hg(II) removal, but large variations observed in removal depending on different background ions suggested that these ions played a role as well. NaCl systems unlike NaNO₃ systems, showed high Hg(II) removal, indicating that Cl was a determining factor in Hg(II) removal. EXAFS data suggested that Hg(II) was finally bound to S in the biochar. We thus propose a stepwise removal mechanism in Cl rich systems: O groups in NOM weakly bound to Hg(II) were competitively displaced by Cl. Smaller sized Hg(II)-chloro complexes were then able to permeate into the biochar pores, where S outcompeted Cl, immobilizing Hg(II) as Hg-S in the biochar. Also, in Ca systems, aggregation and increased hydrophobicity of NOM, including Hg-NOM complexes, promoted Hg(II) removal by biochar. Our results highlighted that NOM is a crucial inhibitor for Hg(II) removal using biochar. However, in saline and Ca rich systems, Hg(II) clean-up can still be effective, even at high NOM levels.

8.04.P-We312 Optimization of Plant Species Composition and Nutrient Additions to Enhance Engineered Floating Wetland Bioremediation of Crude Oil Spills in Freshwater Environments

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The transportation of crude oil creates a risk of accidental spills into freshwater ecosystems. Such spills must be remediated to limit negative effects on local communities and biota. Conventional mechanical and chemical cleanup methods can exacerbate the negative impacts of spills, delaying ecosystem recovery and leaving residual oil in the environment. One minimally invasive alternative is the use of engineered floating wetlands (EFWs) – artificial vegetated platforms which promote microbial colonization and biofilm formation in their underwater rootzones – to enhance biodegradation of oil. In 2021, research conducted at the International Institute for Sustainable Development Experimental Lakes Area, Ontario, Canada investigated the optimization of EFW design for the bioremediation of freshwater oil spills. Two aspects of EFW design were studied: 1) the composition of vegetation species (*Typha* sp., *Carex atherodes*, and *C. lasiocarpa*) planted on the EFW; and 2) the carbon to phosphorus (C:P) ratio resulting from additions of slow-release fertilizer alongside an EFW. Twenty-six mesocosm tanks (1600 L) were exposed to equal-volume spills of water accommodated fraction (WAF) produced by weathering conventional heavy crude oil. Each mesocosm contained an EFW which was either populated with a different ratio of the three plant species or evenly planted with the species and augmented with slow-release fertilizer to achieve a different C:P ratio. WAF was applied in June 2021 and the mesocosms were monitored over 13 weeks for concentrations of 44 polycyclic aromatic compounds (PACs), nutrient chemistry, and the composition and activity of the rootzone microbial community. Preliminary results indicate a rapid reduction in concentrations of sixteen parent PACs in all treatments in the first 35 days of the exposure. Analyses of PAC chemistry and the microbial community will be used to identify EFW design parameters which promote a robust oil-degrading microbial community in the rootzone and enhance the biodegradation of PACs. Ultimately, results from this study will be used to support the application of EFW-enhanced bioremediation as an alternative or supplement to conventional oil spill cleanup techniques.

8.04.P-We313 POPs Home and Away: Targeted PFAS Analysis Using UPLC - MS/MS in UK Cetaceans and Sub-Antarctic Seabirds

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Poly- and perfluoroalkyl substances (PFAS) are a large group of chemicals that are characterised by a hydrophilic moiety (e.g. SO_3^- or COO^-) and a hydrophobic perfluorocarbon chain that varies in length. PFAS display unique properties due to combined oil and water repellence, extreme stability, and hence are used in a wide range of industrial processes and consumer products. These chemicals can be problematic in the environment due to their potential to undergo long-range transport, persistence and toxicity. However, only some of these substances have been regulated by the Stockholm Convention; therefore, there is a growing need to document evidence of these emerging chemicals of concern in the environment. Here, we employ an ultra-performance liquid chromatograph coupled to a triple quadrupole mass spectrometer to analyse a total of 39 PFAS compounds, including 11 PFCAs (C4-C14), 9 PFSAAs (C4-C10), 3 FASAs, 3 PFPiAs and 6 fluorotelomer acids, among others, in the livers of a range of less commonly studied cetaceans sampled over the last decade from around the UK; and in albatrosses and petrels collected from the South Atlantic Ocean from the early 2000s to the mid 2010s. The data are used to assess spatial and temporal trends between different species and compounds; in particular to draw comparisons between trophic levels by employing stable isotope analysis, in seabird species in the sub-Antarctic. Our research addresses the need to understand exposure levels in wildlife to PFAS compounds in areas close to and far away from sources, and also how PFAS fingerprints vary between different species over time.

8.04.P-We314 Quantifying the Wet and Dry Atmospheric Deposition of Microplastics in Switzerland Using Analytical Methods Harmonized Across Environmental Matrices

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Microplastics (MPs) are found virtually everywhere – in the atmosphere, waterbodies, landmasses, mountaintops and at the poles. Organisms of all forms, including humans, are exposed to these MPs. Exposure to atmospheric MPs specifically may have implications for human health as some of these airborne particles are small enough to enter the lungs when inhaled and may cause inflammatory responses. Moreover, atmospheric MPs are transported to locations far from their emission sources and contaminate pristine ecosystems. However, accurate quantification of MPs in different environmental compartments is currently hampered by the lack of harmonized analytical methods. This makes it difficult to compare data generated for MP samples collected from different environmental matrices. We will therefore work towards a harmonized analytical method. The method will use optical microscopy to determine the number of MPs in atmospheric deposition samples and identify their shapes and sizes. Fourier transform infrared spectroscopy, cross-validated with Pyrolysis-Gas Chromatography/Mass Spectrometry, will be used to identify the chemical composition of the MPs. The sampling and analytical processes will be quality controlled using surrogate standards. This work will be done in close collaboration with two other research projects that will investigate MPs in soil and sewage sludge

to harmonize the method across environmental media. The method will be used to monitor the amount and types of MPs in wet and dry atmospheric deposition as collected over a one-year period in urban, semi-urban, rural and remote locations across Switzerland. By doing so, we seek to identify the major emission sources of atmospheric MPs and their relative importance. Gathering this information will be useful for environmental authorities to gain a better understanding of the role of the atmosphere for the distribution of MPs in the environment and also to define appropriate policy measures to prevent or mitigate the effects of MP contamination.

8.04.P-We315 The Assessment of Environmental Risk Related to the Occurrence of Pharmaceuticals in Bottom Sediments of the Odra River Estuary (SW Baltic Sea)

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The Odra River estuary is a north-western region of Poland of high economic and ecological value. The northern part of the estuary is the Natura 2000, a special nature protection area. It is situated at the contact point between freshwater and seawater and has special significance for fish living in these habitats. On the other hand, the Odra River estuary is a highly urbanized and industrialized area with shipyards and two deepwater seaports. The occurrence of pharmaceutically active compounds (PhACs) in the aquatic environment is of great concern due to the potential risk to the ecosystem and human health. The main source of PhACs in the environment is treated municipal wastewaters. Currently, available treatment technologies in wastewater treatment plants (WWTPs) do not remove completely PhACs from wastewaters; thus a certain amount of the compounds remain in the treated effluents which are released to the environment. This study was aimed to find new emerging substances of very high concern that should be covered by surface water monitoring. The occurrence of 130 PhACs in sediments collected from 70 sampling sites in the Odra River estuary was investigated. PhACs were selected after the qualitative screening of sediments with the use of the HPLC system, Dionex Ultimate 3000, coupled with a Q-Exactive hybrid quadrupole-Orbitrap mass spectrometer system (Thermo Fisher Scientific, Waltham, MA, USA), and the literature data. Quantitation of selected PhACs was performed in multiple reaction monitoring (MRM) and positive ionization (ESI+) modes with the use of HPLC, Agilent 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA) equipped with a degasser, thermostated autosampler, and binary pump, and coupled to Hybrid Triple Quadrupole/Linear Ion trap mass spectrometer (QTRAP@4000, AB SCIEX, Framingham, MA, USA). The highest concentration levels of the compounds were found in the vicinity of effluent discharge from two main Szczecin wastewater treatment plants: "Pomorzany" and "Zdroje", and nearby the seaport and shipyard. The high environmental risk was observed in 30 sampling sites for clindamycin, 22 sampling sites for pseudoephedrine, 19 sampling sites for nalidixic acid, 4 sampling sites for carbamazepine, and 3 sampling sites for fexofenadine. Due to the scarcity of toxicological data related to benthic organisms, only an approximate assessment of the environmental risk of PhACs is possible. Nevertheless, the compounds with medium and high risk should be considered as pollutants of high environmental concern whose occurrence in the environment should remain under close scrutiny. The project was financed by National Science Center Poland OPUS 11 (UMO-2016/21/B/ST10/02391)

8.04.P-We316 The Presence of Pharmaceuticals in Our Surface Waters and the Development of Effective Monitoring Strategies

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Pharmaceuticals play a pivotal role in human and animal health. The ever-increasing use and availability of pharmaceuticals in the last decade have led to the contamination of surface water ecosystems from ng/L to µg/L concentrations. The environmental fate and toxicological implications of many pharmaceuticals and their residues remain generally unknown. Additionally, the stability and biological activity of these "micro-pollutants" can lead to a "pseudo persistence" in the environment, with ensuing behavioural and health-related effects. This project highlights the occurrence, movement, and impact pharmaceuticals may have on surface water catchments. Findings from this review will highlight the importance of monitoring pharmaceuticals in surface waters, potential sources and factors that influence their concentrations. Furthermore, detailing strategic monitoring strategies will show the practicality of passive (long-term continuous) and grab (snapshot) sampling techniques in conjunction with effect based biomonitoring tools to give a detailed account of the passage of pharmaceuticals through an aquatic ecosystem. Outcomes from this research will aim to provide information on the relevant concentrations of pharmaceuticals in surface water systems while improving education surrounding appropriate use, disposal and waste management of pharmaceutical products.

8.04.P-We317 Tissue Metal Concentrations and Antioxidant Enzyme Activity in Sharks

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Metals occur naturally in the environment; however, anthropogenic practices have resulted in increased metal concentrations in coastal ecosystems. Sharks are important species, ecologically, recreationally and commercially. Reference levels of metal contaminants in the tissues of sharks, particularly, great whites, is lacking. In this study, concentrations of copper, cadmium, nickel, lead, selenium, silver, and zinc were measured in the muscle tissue of great white and tiger sharks. Metal exposure in various species has been correlated with oxidative stress. Therefore, activities of antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) were also examined in the shark muscle tissue with the objective of identifying a nonlethal bioindicator of metal pollution. Metal-specific differences in tissue metal concentrations were detected based on sex, age class,

and collection site. This study provides new data on metal concentrations in the muscle tissue of great whites collected over the past three years and provides insight into oxidative stress defenses in these top-level carnivores.

8.04.P-We318 Trace Analysis of Pesticides and Assessment of Their Occurrence in MBR Post-Treatment Effluent, After a Pilot-Plant of Potable WATER Reuse and in Drinking WATER

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The water reuse for potable purposes can represent a realistic source for the supply of drinking water in areas with water scarcity. Therefore, it is necessary to combine conventional technologies in the wastewater treatment with advanced technologies for the removal of trace contaminants to have high quality and safe water. The contamination of pesticides is a key point to be addressed in countries like Brazil, where the agriculture business is strong, and the sanitation condition is still poor in many regions. The aim of this work was to validate an analytical method using SPE and LC-MS/MS for the determination atrazine, hydroxyatrazine (ATZOH), desethylatrazine (DEA), ametryn, simazine, diuron, 2,4-D, fipronil, fipronil sulfide and fipronil sulfone in aquatic matrices. The method has been applied to evaluate the concentration of these contaminants in real samples collected from a water reclamation plant and drinking water. All analytes were quantified by internal calibration, except for fipronil and its metabolites, which were quantified by external calibration. The calibration curve determination coefficients (R^2) were at least 0.99 for all analytes. The limits of quantification were between 0.5 and 2.5 $\mu\text{g L}^{-1}$. SPE recoveries were assessed in three levels of concentration (20, 50, and 100 ng L^{-1}) using spiked ultrapure water, drinking water, effluent after membrane bioreactor (MBR) treatment and effluent after advanced treatment. The recoveries ranged between 27 and 141%, with a maximum RSD of 31%. The matrix effect in effluent after MBR, effluent after advanced treatment and drinking water ranged from 3.8 to 59.1%, 6.2 to 20.4% and 3.1 to 27.7%, respectively. Pesticides were quantified in drinking water samples collected in different tap water of the urban city and in a water reclamation plant in two steps: (1) MBR post-treatment and (2) from a pilot-plant of potable water reuse after the follow treatments: reverse osmosis, UV- H_2O_2 and activated carbon. The mean concentration of atrazine, OHATZ, DEA, diuron and fipronil, fipronil sulfide and fipronil sulfone in the MBR post-treatment was 71.2, 49.6, 19.1, 121.0, 43.5, 7.6, 3.6 ng L^{-1} , respectively. After the advanced treatment, only fipronil was quantified with a concentration of 0.7 ng L^{-1} . In drinking water, atrazine, ATZOH, DEA, diuron and 2,4-D were quantified with mean concentration of 33.5, 26.9, 12.1, 8.1 and 138.1 ng L^{-1} , respectively. Therefore, it is possible to conclude that the advanced treatment used in a pilot scale is promising to be applied in a water reclamation plant for potable purpose. However, more studies should be done to classify it as a drinking water.

8.04.P-We319 Designing tools to predict and mitigate impacts on water quality following the Australian 2019/2020 wildfires: Insights from Sydney's largest water supply catchment

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The 2019/2020 Australian bushfires (or wildfires) burned the largest forested area in Australia's recorded history, with major socio-economic and environmental consequences. Among the largest fires was the 280 000 ha Green Wattle Creek Fire, which burned large forested areas of the Warragamba catchment. This protected catchment provides critical ecosystem services for Lake Burragorang, one of Australia's largest urban supply reservoirs delivering ~85% of the water used in Greater Sydney. Water New South Wales (WaterNSW) is the utility responsible for managing water quality in Lake Burragorang. Its postfire risk assessment, done in collaboration with researchers in Australia, the UK, and United States, involved (i) identifying pyrogenic contaminants in ash and soil; (ii) quantifying ash loads and contaminant concentrations across the burned area; and (iii) estimating the probability and quantity of soil, ash, and associated contaminant entrainment for different rainfall scenarios. The work included refining the capabilities of the new WEPPcloud-WATAR-AU model (Water Erosion Prediction Project cloud-Wildfire Ash Transport And Risk-Australia) for predicting sediment, ash, and contaminant transport, aided by outcomes from previous collaborative postfire research in the catchment. Approximately two weeks after the Green Wattle Creek Fire was contained, an extreme rainfall event (~276 mm in 72 h) caused extensive ash and sediment delivery into the reservoir. The risk assessment informed on-ground monitoring and operational mitigation measures (deployment of debris-catching booms and adjustment of the water supply system configuration), ensuring the continuity of safe water supply to Sydney. WEPPcloud-WATAR-AU outputs can prioritize recovery interventions for managing water quality risks by quantifying contaminants on the hillslopes, anticipating water contamination risk, and identifying areas with high susceptibility to ash and sediment transport. This collaborative interaction among scientists and water managers, aimed also at refining model capabilities and outputs to meet managers' needs, exemplifies the successful outcomes that can be achieved at the interface of industry and science. *Integr Environ Assess Manag* 2021;17:1151–1161. © 2021 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

8.04.P-We320 Zebrafish as a model to screen Thyroid Disruption

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There is an increasing concern about the environmental impacts of chemicals that can disrupt the endocrine system, which can result in adverse effects on developmental, reproductive, neurological, and/or immune functions in both humans and wildlife. The negative impact of these Endocrine Disrupting Compounds (EDCs) is becoming a real public health issue, therefore the necessity of tests to assess the potential risk of new chemicals before they are marketed is increasing. In vitro tools are inexpensive and

scalable for high-throughput platforms, however, they pose low relevance to vertebrate species. On the other hand, working with adult animals is expensive and represents ethical issues. Small fish like zebrafish (*Danio rerio*) are an excellent alternative to in vitro and in vivo models. They offer a unique experimental system where screening assays can be performed at the whole animal level, being at the same time compatible with the 3R principles (Replacement, Reduction, and Refinement in animal testing). In this work, we present a three-phase assay to screen the endocrine disrupting potential of chemical compounds. First, thyroid disrupting (TD) chemicals are evaluated using the transgenic line tg(tg:mcherry), which expresses red fluorescence in the thyroid gland under the thyroglobulin promoter. The fluorescence of the reporter gene allows monitoring in vivo the upregulation of the thyroglobulin gene expression as a compensatory reaction to thyroid gland disruption. The signal intensity is assessed in vivo to screen and characterize potential TD, either directly or after metabolization by the zebrafish embryo. Second, a gene expression analysis is carried out to characterize the thyroid disrupting effect by measuring three thyroid-related genes (*tsh?*, *tg*, and *tpo*). And third, the thyroid hormone (TH) levels of T4, T3, 3,5-T2, and 3,3'-T2 are quantified by liquid chromatography (LC/HRMS) in whole-embryo extracts. Our data show that reference compounds (such as potassium perchlorate and propylthiouracil), and environmental pollutants (such as benzophenone-2) induce an increase of the fluorescence of thyroglobulin in the tg(tg:mcherry) transgenic line, and overexpression of thyroid-related genes *tsh?*, *tg*, and *tpo*. These results were confirmed in TH level measurements by LC/HRMS. This screening methodology showed to be a sensitive and cost-effective assay to screen and evaluate potential EDCs chemicals.

8.04.P-We321 Risk Reductions During Biotransformation and Mobilisation of Pyrene in a Model Plant-Bacteria-Soil System

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The productive application of motile microorganisms for degrading soil contaminants is one of the most promising processes in modern remediation due to its low cost and efficiency. However, the incomplete biodegradation of the pollutants and the formation of the intermediary metabolites in the process, may increase the toxicity in soil during bioremediation (*Environ. Sci. Technol.* 12: 421-444, 2013; *Sci. Total Environ* 689: 390-397, 2019). Therefore, controlling these factors should be a fundamental part of soil remediation approaches. The aim of this study was to evaluate the sources of risk associated with the transformation by cometabolism of ¹⁴C-labelled pyrene by inoculated *Pseudomonas putida* G7, and identify ways for risk minimization. Our model scenario tried to increase bio-accessibility to a distant source of contamination through the chemotactic bacteria attraction exerted by sunflower (*Helianthus annuus* L.) root exudates. A biochar trap for mobilized pollutant metabolites and bacteria has also been employed. The experimental design consisted of pots comprising a layer of sand with ¹⁴C-labelled pyrene (88 mg/kg) placed approximately at mid-height as contamination source. Half of the pots included a biochar layer at the bottom. The pots were incubated in a greenhouse with sunflower plants and *P. putida* G7 bacteria (*Sci. Total Environ* 760: 143408, 2021). The results showed that those pots with sunflower plants evidenced a higher bacterial mobilisation towards the source of contamination by chemotaxis, resulting in the biodegradation of pyrene and its mobilization as metabolites through the percolate and the roots. In addition, the biochar layer efficiently reduced the concentrations of pyrene metabolites collected in the leachates. Therefore, the combination of plant, bacteria and biochar safely reduced the risks caused by the biological transformation. Although this is a preliminary greenhouse-scale model, progress in this field will undoubtedly open up new possibilities for low-cost control and treatment of contaminated soils.

8.04.P-We322 Chemotactic Pseudomonas Putida G7 Facilitates the Co-Mobilization of Immotile Pollutant-Degrading Bacteria in Restricted Porous Regimes

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Dispersal of degrading microorganisms within porous media is one of the key drivers of bioremediation of heterogeneously polluted environments (*Curr Opin Biotechnol* 24, 451–456, 2013). Recent studies have identified a unique form of bacterial dispersal by hitchhiking on motile microbes (*Trends Microbiol* 29, 542–550, 2021). The dispersal enhancement and co-motility of immotile cells of a HCH-degrading bacterial strain, *Sphingobium* sp. D4, in the presence of a chemotactic and naphthalene-degrading strain, *Pseudomonas putida* G7, was investigated using glass bioreactors. They comprise two chambers separated by membrane filters of different micrometer-sized pores (3, 5 and 12 µm): the lower chamber (LC), where cells were injected, and the upper chamber (UC), where transported cell quantification was performed. Chemoeffectors in the UC (sodium salicylate, a naphthalene degradation product, or γ -aminobutyric acid–GABA-, a common component of root exudates) induced the tactic motility of G7 in the direction of a positive concentration gradient. D4 cells were transported to UC by means of two fluxes: Brownian dispersion and co-mobilization through the G7 tactic front. These fluxes changed in intensity with time: Brownian dispersion was the main mechanism within the first 1-2 h, while G7 co-mobilization was more significant after 4-6 h, when the maximum G7 transport to the UC occurred. After 8 h (initial cell rate 1:1, D4/G7), ca. 6% and 7% (using salicylate), and ca. 13% and 16% (using GABA) of D4 initial cells were co-transported to UC, using 5 and 12 µm membrane pore sizes, respectively. Only 3% (salicylate) and 7% (GABA) of D4 cells were mobilized in the absence of G7. A higher proportion of initial G7 cells (1:10, D4/G7) lead to a higher co-mobilization: e.g., 9% of initial D4 cells were mobilized to UC in 5 µm-membrane bioreactors. Microscope observations confirmed that the main mechanism for D4 hitchhiking was mechanical pushing by the G7 front, due to cell collisions and the turbulences generated: neither multimicrobial aggregates were observed nor direct attachment to G7 cells,

dismissing those mechanisms under our experimental conditions. The results reflected the potential of chemotactic microbes for enhancing the dispersal of immotile microorganisms in restricted pore systems. Carefully selected bacterial consortia (degrader/s+carrier/s) could, therefore, significantly enhance the accessibility to contaminant sources in remediation procedures.

8.04 Extended submission THU (Poster)

8.04.P-Th213 A Complex Mixture of Polyaromatic Compounds From a Contaminated Soil Cause Embryotoxic Effects and Alter the Behavior and Gene Expression in Zebrafish Embryos (*Danio rerio*)

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Environmental contamination is usually comprised of a complex mixture of pollutants, and each of them has the potential of causing different toxic responses towards humans and wildlife. One approach to study complex mixtures in environmental samples is to use effect-directed analysis (EDA), combining fractionation, bioassays, targeted and non-targeted chemical analysis. In the present study, toxicity tests with zebrafish embryos (*Danio rerio*) were performed to investigate acute toxicity, molecular, and adverse effects of an extract from a contaminated soil collected at a former gasworks site contaminated with polyaromatic compounds (PACs). For this purpose, three replicates were conducted in 96-well plates, and each replicate consisted of 192 individuals divided into five concentrations and three control groups. The chemical composition and distribution were determined by measurements of the concentrations of 87 PACs (including polycyclic aromatic hydrocarbons (PAHs), oxygenated PAHs, alkylated PAHs, and heterocyclic PACs (NSO-PACs)) in the water phase, the fish, and sorbed to the plate material. Based on the data for acute toxicity and sub-lethality, gene expression and behavioral alterations were investigated to study the underlying toxic mechanisms. The zebrafish behavior was measured as larvae movement during exposure to interchanging light and dark periods. In addition, the response to mechanical stimuli was recorded using a tapping device. Quantitative gene expression analyses were conducted on the set of 36 selected genes that are known to be directly linked to certain adverse outcomes. The complex mixture was acutely toxic to the embryos and the exposure resulted in several teratogenic effects as divided yolk-sac, edema on the heart, and reduced pigmentation. In addition, the mixture altered the behavior of the larvae and caused hyperactivity in darkness and hypoactivity in light. The larvae were also hypoactive after the mechanical stimuli. The qPCR analysis showed that eleven genes were differentially regulated. For instance, one gene connected to pigmentation in the eye (*opn1sw1*) was downregulated and a gene involved in cardiomegaly and heart failure (*fpgs*) was upregulated. In the next step of this study, an EDA is performed with the extract to identify (novel) PACs or other organic compounds causing the observed toxic effects. The results will increase the knowledge of PACs present at contaminated sites, their identity, and toxicity.

8.04.P-Th214 A New Oil Spill Dispersant Developed According to Efficacy and EcoToxicological Criteria

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Dispersants are specially designed oil spill mixtures that are composed of detergent-like surfactants in a variety of organic solvents. Dispersants do not remove oil from the water, but instead break the oil into small droplets. In Argentina, the environmental legislation authorizes the applications of dispersant formulations based in three characteristics: efficacy, biodegradation and ecotoxicity. Based on this, we designed a dispersant mixture taking the following components: 2-Butoxy-Ethanol, Polisorbate 80/60, Span80, Tween80, Ethanol, lecithine and diocetilsulfosuccinate. A combination of design of experiments (DOE) using a Central Composite Design (CCD) and Mixtox models for ecotoxicity was used to test all dispersant formulations. Efficacy was evaluated by Barrel shaker method, biodegradation by OECD 301 method and ecotoxicity was evaluated on one fish species of Poeciliidae (*Cnesterodon decemmaculatus*), *Artemia franciscana* and *Metacyclops gracilis* a widespread hyporheic copepod species. The new dispersants were applied on the follow types of petroleum extracted in Argentina wells: Caleta Córdoba, Caleta Olivia, Hydra Total and Cruz del Sur – YPF. And were compared with the response of Corexit 9500A and 9527A. Final formulations were also characterized for Interfacial tension and droplet sizes distribution. A discussion is made about how changes in proportions of each component determines the % of Efficacy and drop interactio forces; also % of biodegradability and ecotoxicity. A final formulation is proposed as fourth generation dispersants.

8.04.P-Th215 Acute Toxicity of the Tire Rubber-Derived Chemical 6ppd-Quinone to Four Fishes of Commercial, Cultural, and Ecological Importance

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Stormwater runoff from urban landscapes has recently been linked to mass mortalities of coho salmon on the U.S. West Coast, also dubbed urban runoff mortality syndrome (UMRS). The chemical responsible for UMRS has been identified as *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone), a transformation product of the rubber tire antioxidant 6PPD, with a median lethal concentration (LC₅₀) <0.1 µg/L for coho salmon. Interestingly, subsequent studies have failed to confirm comparable sensitivity in a few other select fish species including chum salmon, medaka and zebrafish, highlighting an urgent need for assessing the potential hazard of 6PPD-quinone to fishes in general. Here, we investigated the acute toxicity of 6PPD-quinone to four fishes of commercial, cultural, and ecological importance in North America, rainbow trout, brook trout, Arctic char, and white sturgeon. Fish were exposed under static renewal conditions and exposure concentrations were verified analytically. Mortalities in brook trout occurred between 1.2 and 20 h, while mortalities began after 7 h and spanned 60 h in

rainbow trout. The LC₅₀s in brook trout (24 h) and rainbow trout (72 h) were 0.87 and 1.00 µg/L, respectively. Both species showed characteristic symptoms (increased ventilation, gasping, loss of equilibrium) shortly before death. No mortalities were observed after 96 h of exposure for either char or sturgeon at measured concentrations as high as 14.2 µg/L. This is the first study to demonstrate acute toxicity of 6PPD-quinone to other fishes of commercial, cultural, and ecological importance at environmentally relevant concentrations and will provide urgently needed information in support of environmental risk assessments of this contaminant of emerging concern.

8.04.P-Th216 Aged NanoPlastics for Laboratory Testing

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Reference particle models are essential to obtain an accurate perspective of how environmental nanoplastics behave in natural systems and to generate data on their environmental fate and impact on living organisms. However, although the current available models are particularly important for filling the initial knowledge gaps on nanoplastics, they do not demonstrate enough diversity and/or accuracy to represent the actual heterogeneity of the physical and chemical properties of environmental nanoplastics. In this framework, the Joint Research Centre (JRC) and the National Institute of Standards and Technology (NIST) are collaborating on a challenging project, which aims to identify a novel strategy for the production of aged nanoplastics. These new test materials are designed to become environmentally relevant models made by the combination of mechanical abrasion and artificial UV exposure for ageing. The objective is to obtain nanoplastics, which mimic naturally weathered ones in size distribution and surface chemistry. The scalable production of these nanoplastics is investigated to make them available to stakeholders. This test material will have applications for the development of new analytical methodologies to detect nanoplastics, to better understand their role in carrying other pollutants and increase knowledge related to their behaviour and fate in the environment.

8.04.P-Th217 Algae Wracks on Coastline of Baltic Sea As Potential Accumulation Points of Microplastic Particles

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Plastics have become an essential part of our everyday life since the beginning of their mass production in the 1940s. With the increasing world population, the demand for plastic products is rising. The predicted increase of plastic production will reach 33 billion tons by 2050. As a result, the amount of plastics (and microplastics) that enter the surrounding environment is increasing. Due to this, coupled with an estimated half-life at sea between 1.4 and >2500 years, plastic pollution in oceans and seas is recognized as a severe environmental issue. It is also the reason for importance to obtain more information on the way it is transported and places of possible accumulation, including beach-cast algae on the sea coastline. The aim of this study is to assess potential of algae wrack in shoreline to be an accumulation spots of microplastic as well as try to define whether macrophyte algae species composition have impact on microplastic amount in algae wracks. In order to reach scientific targets of the study in July, August and September of 2021 sampling was performed in the Latvian coastline of Baltic Sea and Gulf of Riga. In eight places samples for microplastic detection in algae wrack, control sand and algae wrack samples were taken. Microplastic sample preparation from algae wracks included several steps including treatment with sodium hydroxide (10 %), peroxide (15%), enzymes (protease, cellulase, viscozyme) and two steps of density separation (SPT - 1,75 g/ml). Control sand sample treatment included only density separation (SPT - 1,75 g/ml). For both microplastic sample matrices final step is filtration on GF/C filters for visual identification under the microscope and analysis. All particles, excluding fibers, that could be handled manually were picked up for further chemical composition analysis. Thereafter, these particles are analysed with the ATR-FTIR spectroscopy method. For macrophyte algae species composition samples were analysed by sorting the macroalgae according to species, which are detected by morphological characteristics as well as dry weight of each species was detected. Preliminary results show that algae wracks in coastline of Baltic sea have potential to accumulate of microplastic particles. Research was performed in the framework of EU Interreg Latvia-Lithuania project "Estimation, monitoring and reduction of plastic pollutants in Latvian-Lithuanian coastal area via innovative tools and awareness raising" (LLI - 525)

8.04.P-Th218 An Agent-Based Model of Epigenetic Mechanisms to Mitigate Environmental Stressors

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Identifying how organisms respond to environmental stressors remains of central importance as human impacts continue to shift the environmental conditions for countless species. Some mammals are able to mitigate these environmental stressors at the cellular level, but the mechanisms by which cells are able to do this and how these strategies vary among species is not well understood. At the cellular level, it is difficult to identify the temporal dynamics of the system through empirical data because fine-grained time course samples are both incomplete and limited by available resources. To help identify the mechanisms by which animal cells mitigate extreme environmental conditions, we propose an agent-based model to capture the dynamics of the system. In the model, agents are regulatory elements and genes, and are able to impact the behaviors of each other. Rather than imposing rules for these interactions among agents, we will begin with randomized sets of rules and calibrate the model based on empirical data of cellular responses to stress. We will apply a common-garden framework to cultured cells from 16 mammalian species, which will yield genomic data and measures of cell morphology and physiology when exposed to different levels of temperature, glucose, and oxygen. These species include humans, dolphins, bats, and camels, among others, which vary in how they respond to environmental stressors, offering a comparative approach for identifying mechanistic rules whereby cells achieve

robustness to environmental stressors. For calibration of the model, we will iteratively select for rules that best lead to the emergent outcomes observed in the cellular assays. Our model is generalized for any species, any cell type, and any environmental stressor, offering many applications of the model beyond our study. This study will increase our understanding of how organisms mitigate environmental stressors at the cellular level such that we can better address how organisms are impacted by and respond to extreme environmental conditions.

8.04.P-Th219 An Optimized Method for the Identification of Ciguatoxins in Fish Using High Resolution Mass Spectrometry

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Ciguatera fish poisoning is a major health issue affecting food sustainability and security for populations located in tropical and subtropical regions, primarily in the Pacific and Caribbean regions. This neurological, foodborne illness is caused by consuming fish that has been contaminated by ciguatoxins (CTX). CTX are a class of toxic polyethers that are similar in molecular structures (analogues) produced by marine dinoflagellates in the genera *Gambierdiscus* and *Fukuya* and bio-transform throughout the food web. Low-resolution mass spectrometry is the most common and preferred method for ciguatoxin identification in fish, however, there is limited standard availability for this approach and it does not allow for the study of the complete array of ciguatoxin analogues that can also contribute to the toxic levels for consumption of the fish. The development of a sensitive method using High-Resolution Mass Spectrometry (HRMS) could be used to help unambiguously identify all CTX analogues even when analytical standards are restricted due to the spectrometer's increased mass accuracy. We have altered and evaluated the changes in four parameters (solvent makeup, voltage, collision energy, resolution) that influence ionization and ion transmission to observe and optimize the behaviour of the sodium and ammonium adducts, protonated molecules, and water losses of CTX-3C on the electrospray ionization source coupled with the Orbitrap mass spectrometer. We will analyze a store-bought fish fillet spiked with the CTX-3C standard to test for the optimized method through the confirmation of the presence of CTX-3C. This optimized method will be further evaluated for identifying numerous CTX analogues by observing similar fragmentation patterns to CTX-3C in a contaminated Caribbean fish supplied to us from the Caribbean Public Health Agency (CARPA). The advantages of having a sensitive HRMS method for CTX identification will allow confirmation of CTX in fish despite having limited standards available, and the further discovery of new CTX analogues that have not been identified. This method may give us new opportunities to delve deeper and gain a greater insight into the fate and cycling of CTX so that we can implement better protective measures against ciguatera fish poisoning.

8.04.P-Th220 Analytical Challenges in Analysis of Pharmaceutical Residues From Sewage Sludge and Soil Samples

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Active pharmaceutical ingredients (APIs) are a chemically diverse group of compounds that end up to the environment largely due to the proper use of medicines. At wastewater treatment plants, APIs are either degraded, retained to sludge or released into recipient waters via effluent wastewater. In Finland, most of the nutrient rich sludge is used in agriculture or landscaping, and especially persistent APIs and their metabolites can end up in soils. Before reuse, the sludge is commonly digested or composted, but it may also be chemically treated, pyrolyzed to biochar or incinerated to ash. Reliable analytical techniques to detect APIs from soil, sludge, biochar or ash samples has to be developed to assess the occurrence and environmental risk caused by these compounds. In general, tens or even hundreds of APIs and their metabolites having differing chemical characteristics has to be determined in one sample, which brings challenges to analytical methods. SUDDEN (Sustainable Drug Discovery and Development with End-of-Life Yield) is a multidisciplinary project studying e.g., emissions, life cycle and ecological risk assessment of certain APIs. In SUDDEN project we optimized pre-treatment, purification and analysis technique to analyse 74 APIs and 12 metabolites from soil, sludge, biochar and ash samples by taking account the chemical characteristics of each compound. The APIs were divided in different groups with similar chemical characteristic and the optimization was conducted for each group. Analytical challenges deriving from complex matrix were tackled with optimizing cleaning processes which were based on solid-liquid extraction followed by solid phase extraction, but also with adjusting chromatographic separation. Basic and acidic conditions were used in both cleaning and liquid chromatography tandem mass spectrometry (LC-MS/MS) runs. High matrix effect was observed in soil and sludge samples. Ash and biochar samples were less complex, presumably due to their lower organic matter content. However, sorption of the analytes to the ash and biochar materials reduced their recoveries remarkably. The developed methods will be used in the SUDDEN project to produce novel information on the occurrence of APIs and their selected metabolites in sludge-based fertilizers and in sludge-amended soils. The work will help identify substances causing the highest risks in the soil environment.

8.04.P-Th221 Analytical Techniques and Associated Challenges in the Determination of Trifluoroacetic Acid and Other Very Persistent and Very Mobile Substances in Aqueous Environmental Samples

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Interest in analyzing small and polar molecules has increased over the past decade. This is a result of the increased attention on substances that are considered persistent, mobile, and toxic (PMT), or very persistent and very mobile (vPvM), due to their expected accumulation in the water cycle. A well-known example is trifluoroacetic acid (TFA), which has been found in environmental samples around the world. Recent attention to substances such as TFA has resulted in the emergence of analytical techniques suitable for the analysis of polar molecules, such as mixed-mode liquid chromatography (MMLC), hydrophilic

interaction liquid chromatography (HILIC), and supercritical fluid chromatography (SFC). The analysis of polar molecules is often hampered by matrix effects due to co-elution with inorganic ions, and mass-labelled standards are not often available. Blank contamination is a common challenge associated with the analysis of TFA, and large variations in recovery during extraction by weak anion exchange solid-phase extraction (WAX-SPE) have been observed. The aim with the present study was to compare different analytical methods for the analysis of TFA and other PMT/vPvMs in aqueous samples, and to address some of the associated challenges. Chromatographic separation of TFA and other PTM/vPvMs was tested using SFC, MMLC, and HILIC. A method based on direct injection analysis with SFC coupled to tandem mass spectrometry after 1:1 dilution in methanol was applied for the determination of TFA in various water samples ($n = 33$). The method was evaluated and compared to a method based on WAX-SPE in terms of method performance. All of the chromatographic techniques tested were shown to be suitable or potentially suitable for the separation of TFA and related substances. While large variations in extraction recovery of TFA (7–42 %) was observed by WAX-SPE in selected test samples ($n = 3$), the recovery by direct injection (due to ion signal effects), was consistent (81 ± 0.4 %). The observed concentrations were up to 600 times lower by WAX-SPE, and the detection frequency was 30 %, compared to 61 % by direct injection. Direct injection using SFC was shown to be advantageous in terms of method performance and is suitable for the analysis of TFA in samples in which the concentration meets the relatively high detection limit. It is a fast and cost-effective method which may be further optimized to include other target analytes of interest.

8.04.P-Th222 Are PMOCs Less Toxic? Linking Physicochemical Compound Properties With Measured Toxicity

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Persistent and mobile organic chemicals (PMOCs) are gaining attention as a threat to the quality of water resources. The high mobility (i.e. polarity) of these chemicals allows them to pass through subsurface environments and evade sorption-based water treatment processes. Their persistence means that (bio)degradation processes have only a very limited effect on their concentrations throughout the water cycle. This leads to the presence and likely accumulation of PMOCs in water that may also act as a source for drinking water production. Yet, given the only recent attention to this vast group of compounds and the analytical challenges that researchers face, little is known about their toxicity. As a result, despite the growing body of environmental concentration data, risk assessment of PMOCs remains in its infancy. To address this knowledge gap, the present study aimed to gain insight into the toxicity and toxic mechanisms in relation to the physicochemical properties that determine the persistence and mobility of chemicals in the environment. The high polarity of these compounds may mean that they interact less with tissues, cell membranes, and receptors than their lipophilic counterparts, leading to lower compound toxicity. Therefore, it was hypothesized that the mobility of PMOCs makes them inherently less toxic than non-polar compounds. To test this hypothesis, a dataset was compiled matching physicochemical data for 3360 water-relevant compounds with their measured effects in 534 unique toxicity tests from the ToxCast program. A random forest analysis identified the physicochemical properties that relate most strongly to the induced effects and regression analyses quantified the strength and direction of these relationships. This indeed showed that compound properties related to polarity, particularly K_{OW} and K_{OC} , are inversely related to effect concentrations, confirming that more polar compounds are, generally, less toxic. The mechanistic clustering of the toxicity tests allowed the derivation of trends and mechanisms explaining the observed results. Although PMOCs appear to be inherently less toxic than more hydrophobic compounds, their diversity and pervasiveness in water cycles warrant further investigations into their potential threat to water quality. The here acquired mechanistic understanding of the link between compound properties and toxicity can aid the (environmental) health risk assessment of PMOCs to safeguard valuable water resources.

8.04.P-Th223 A Comparison of Species Sensitivity Distributions (SSDs) for Fish and Aquatic LIFE Stages of Amphibians

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Acute fish testing is a standard data requirement for crop protection product registration. The fish acute test yields a 96-h LC50 which is used with an assessment factor (AF) of 100 in the EU aquatic risk assessment (RA). A common refinement option for the acute RA is the species sensitivity distribution (SSD), a cumulative distribution fitted to at least 5 LC50 values from different fish species. Then, the hazardous concentration for 5% of species (HC5) is derived from the SSD and used in the RA with an AF of 9. There is no requirement to test aquatic life stages of amphibians, but there is a requirement to assess available data (e.g., from public literature). The common approach is to assume that fish cover the sensitivity of the aquatic life stages of amphibians, which has been confirmed by several data reviews (EFSA, 2013; Weltje et al., 2013). However, it has also been suggested to increase the AF further to include all retrieved data points from the public literature (Ortiz-Santaliestra et al., 2018). Additionally, the scientific opinion on RA for amphibians and reptiles (EFSA, 2018) urges to perform toxicity tests with aquatic amphibian life stages. However, this suggestion clearly contradicts the wish to reduce animal testing as per regulation EU 1107/2009. Finally, the AGD (EFSA, 2013) states that it should be checked whether a refined fish RA (e.g., with an SSD) still covers the aquatic amphibian life stages. Therefore, we constructed SSDs for fish and amphibians for 15 insecticides and fungicides, using active substance and formulation data. Herbicides were not included due to (known) formulation effects. LC50 values for amphibians and fish were obtained from the U.S. EPA ECOTOX database, supplemented with scientific and regulatory literature. SSDs were calculated for fish and amphibians separately and for the combined dataset to investigate 1) sensitivity differences and 2) if fish and amphibians are part of the same aquatic vertebrate SSD. Our analyses suggest that fish and amphibians are indeed part of the same aquatic vertebrate SSD, and that fish are often more sensitive than amphibians. **References** EFSA 2013. *EFSA J*, 11, 3290EFSA 2018. *EFSA J*, 16, e05125Ortiz-Santaliestra et al., 2018. *Ecotoxicol*, 27, 819 Weltje et al., 2013. *Env Toxicol Chem*, 32, 984

8.04.P-Th224 Behavioral Alteration in Zebrafish Following Early LIFE Exposure to Perfluoroalkyl Substances

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Poly- and perfluoroalkyl substances (PFAS) have received worldwide attention due to their persistent, toxic and bioaccumulative properties. PFAS were used extensively in firefighting foams, textiles, and paper products and can nowadays frequently be detected across all environmental matrices. Among PFAS, perfluorooctane sulfonic acid (PFOS) is the most studied compound. Among other effects, it has been shown to cause developmental toxicity and neurotoxicity in many different vertebrate species including zebrafish. Present research found that PFOS accumulates in the brain, hinders neuronal growth, and causes behavioral alterations. These alterations include hyperactivity in stressful situations. Perfluorobutane sulfonic acid (PFBS) is now often used as a replacement for PFOS, since it's considered as less toxic and bioaccumulative. However, preliminary own results show an adverse effect on an individual level of PFBS. However, the effect of PFBS on the nervous system of fish and related alterations of the behavior with direct consequences on the survival remains unknown. Therefore, the major aim of this project is to investigate behavioral alterations of zebrafish (*Danio rerio*) at different early life stages caused by PFOS and PFBS. Zebrafish embryos were exposed from 1 hour post fertilization (hpf) to 28 days post fertilization with two concentrations of PFOS and PFBS. The behavior of zebrafish larvae was investigated at 120 hpf and 144 hpf using a larval photo-motor response analysis (LPMR) and a vibrational stimulus (VS) test. At adult stages, anxiety response was analyzed with a novel tank diving test (NTT). The results indicate a concentration dependent effect of PFBS, though this should be validated in future projects. Moreover, PFOS induces a reduction in fish mobility when faced to a stimulus mimicking a predator presence (light off) and an anxiety increase in response to tapping stress. Our results show that PFOS and PFBS alter the behavioral responses of zebrafish larvae in the presence of predators or in stressed situations. The NTT test is still ongoing. With our results we will decipher further the understanding of the toxicity of both chemicals and their adverse effect on behavior.

8.04.P-Th225 Bioaccumulation of Pharmaceuticals and Endocrine Disrupting Chemicals in Macrophytes

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Pollution with sewage effluents causes the presence of various emerging contaminants, like pharmaceuticals (PhACs) and endocrine disrupting chemicals (EDCs), in surface waters worldwide. Their environmental fate and effects on aquatic biota are still largely unexplored. Macrophytes, aquatic plants and mosses, are often used in phytoremediation experiments, due to their ability to accumulate various contaminants from the environment. Macrophytes could, therefore, potentially act like storage units for some of the PhACs and EDCs and could reintroduce them into environment through food webs. In order to examine the bioaccumulation patterns of the PhACs and EDCs in macrophytes, we conducted a field study on five rivers in Croatia. We selected 10 sampling sites to represent effluent impacted watercourses and clean controls. In addition to macrophytes, we also sampled water and biofilm at each site. Samples were screened for contaminants using an ultra-performance liquid chromatography (UPLC) system coupled to a quadrupole time-of-flight mass spectrometer. A total of 23 compounds were detected, mostly additives and industrial chemicals. Water samples from impacted sites had generally highest numbers of detected compounds, followed by macrophyte samples, whereas lowest number was detected in biofilm samples. We also aimed at testing the potential variation of PhACs and EDCs bioaccumulation patterns between mosses and higher aquatic plants, however, preliminary results indicate that there are no differences. Furthermore, in order to assess possible differences in distribution of accumulated PhACs and EDCs in different parts of aquatic plants, roots and leaves were analysed separately. Similarly, lower (older) and upper (new) shoots of mosses were analysed separately. Nevertheless, there were no clear trends in number of compounds detected in different parts of both macrophyte groups. The current study contributes to providing understanding on the role of primary producers, such as macrophytes, in accumulation of PhACs and EDCs in aquatic environments.

8.04.P-Th226 Biodegradation of Weathered PE and Bioplastic Films in Seawater

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It is widely documented, that plastic debris is present in the marine environment: from coastal areas to the open ocean, from surface to seabed. It has been estimated that millions of tons of plastic waste enters the ocean due to waste mismanagement. Floating plastics, and beached plastic litter, are continuously exposed to UV light, causing surface alterations. Also, biofilms are formed through the attachment of natural marine bacteria and other microorganisms. In this study, we have investigated the biodegradation of plastic films in seawater: pristine polyethylene (PE) films, UV-weathered PE films and bioplastic (starch blend) films. UV weathering was performed using an ATLAS Suntest XLS+ chamber, with a cycle of flooded and dry conditions, to imitate tidal waters. Material characterization of the UV-weathered PE films in the SEM and FTIR showed oxidative degradation by formation of cracks and oxidized functional groups. Formation of anhydrides as reaction product of carbonyl groups suggests a high degree of degradation for the UV-weathered PE compared to the pristine material. Biodegradation tests were carried out following ISO 23977-2:2020 with modifications based on OECD TG306F. The tests were performed using an Oxitop® closed bottle system incubated for 28 days at 20 deg C. Biodegradation was investigated in natural seawater (collected at Kristineberg, Sweden) as well as in seawater with addition of marine bacteria isolated from plastics (bioaugmentation). The biological oxygen demand (BOD) was monitored based on the generation of CO₂, absorbed by NaOH pellets, and measured as a decrease in pressure inside the bottles. The biodegradation was then expressed as BOD in percentage of Theoretical Oxygen Demand (ThOD). Results demonstrate a microbial activity of the seawater, sufficient to degrade a known biodegradable reference material (cellulose). Biodegradation further increased with bioaugmentation. Bioplastic films were found to degrade to a larger extent than PE films. A low degree of PE degradation was observed during the 28 days period, independent of UV weathering. This study

demonstrates the feasibility of a standardised test setup for degradation testing, with the purpose of obtaining insights into the parameters governing biodegradation of plastics in the marine environment.

8.04.P-Th227 Biological Assessment of Pesticide Effects at Swedish Monitoring Sites

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For sustainable management of agricultural landscapes there is a need to evaluate biological effects of pesticides. In this study, monitoring data from 2010 to 2019 from four national monitoring streams in Sweden were evaluated. Pesticide concentrations in water samples (weekly averages of 100 subsamples) and biological samples, benthic invertebrates and benthic diatoms (yearly samples), were evaluated. Based on these data, and existing toxicity data from the Pesticide Properties Database, biological indicators were calculated to estimate the impacts on aquatic ecosystem. The results show that a relatively diverse community of invertebrates occur in the streams, on average 23-30 species. However, there was a significant decrease of number of species at the two sites in central Sweden between 2010 - 2019 (Mann-Kendall trend test $p < 0.05$). Proportions of deformed valves of diatoms (%) showed for some year's exceedance of the 2% effect level, which indicate effects of heavy metals or pesticides. Calculated eutrophication indexes for both algae and invertebrates showed typical nutrient-rich conditions for all sites. Values for the benthic invertebrate index SPEcies At Risk (SPEAR) ranged between 0 – 0.18 at all sites, which means the amount of sensitive species compared to tolerant species is rather low. The values show greater impact of benthic invertebrates at the four sites compared to other streams in southern Sweden. The Pesticide Toxicity Index (PTI) sums up detected concentrations of pesticides, here up to 120, in surface water and are compared to their toxicity levels. PTI showed higher values for the two sites in the south of Sweden compared to the two sites in central Sweden, indicating larger effects of pesticides. An explanation is that the amount of pesticides used in the south of Sweden is higher, due to e.g. warmer climate and the use of crops with intensive pesticide use. No specific time trends or correlations could be seen for the PTI and SPEAR index. The indexes indicate that the four monitoring sites are affected by nutrients as well as by pesticides. The indirect assessment of biological effects of pesticides using indexes such as proportions of deformed valves of diatoms and SPEAR can help to understand integrated effects over time and quantifying more directly effects on organism communities.

8.04.P-Th228 Biotransformation of Phenylarsenic Chemical Warfare Agents in Marine Environment

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After World War II, extensive dumping operations of chemical weapons (CWs) took place in the Baltic Sea area and the Skagerrak Strait, where the loads of dumped CWs were approximately 50,000 and 170,000 tons, respectively. Abandoned CWs containing toxic chemical warfare agents (CWAs) have raised concern not only for environmental but also safety reasons. Studies have shown that corroded munitions containing toxic CWAs are leaking and therefore causing a risk to the marine environment. Especially phenylarsenic chemicals, such as Adamsite and Clark-related chemicals, are of great interest due to their bioaccumulative potential and toxicity in marine organisms. Phenylarsenic chemicals have already been found in marine biota species living in the vicinity of different dumping areas. The ongoing the WARTOX project (2021-2023) funded by the Academy of Finland investigates the mechanisms of phenylarsenic CWA biotransformation in Baltic Sea sediments and their toxicity. The project aims to characterize the main microbial groups responsible for the formation of biotransformation products in the sediment and to develop targeted chemical analysis methods for the identified compounds, in order to assess the total burden of phenylarsenic CWAs in marine sediment. Previously unreported degradation products of phenylarsenic CWAs, such as sulfur-containing and methylated compounds, have been found in sediment samples collected from the known dumping sites. In the WARTOX project, studies now show that the microbial groups present in the sea sediment are impacting the transformation of CWAs to these novel, sulfur-containing compounds. Similarly, the sediment microbiota may be responsible for the production of methyl-containing derivatives of the dumped CWAs. In the framework of the WARTOX project, the toxicity and sublethal effects of the these novel biotransformation will be assessed, utilizing model aquatic species. The effects of CWA-related phenylarsenic chemicals on marine biota is still poorly understood as well as their environmental behavior is unknown. The information on the bioaccumulation of CWA-related phenylarsenic chemicals, their metabolism, toxicity, and biotransformation products present in the marine environment is essential for enhanced risk assessment related to sea-dumped chemical munitions.

8.04.P-Th229 Can Bacterial Growth Be Used As an Indicator for Biodegradation of Chemicals in a High Throughput Screening Test for Readily Biodegradability?

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Standard biodegradation screening tests (BSTs) are laborious and time consuming, thus limiting the number of chemicals that are feasible to test in the laboratory at the same time. Modelling tools, such as BOWIN for predicting biodegradation, have limited applicability domains. In the current project, we identified a list of 250 chemicals in Norway that should be prioritized for evaluating endocrine disrupting properties and environmental risk assessment. A high throughput screening test that would be applicable for water soluble and non-volatile down the drain substances and that can be adopted to automation is warranted to test the biodegradation potential of many chemicals. Standard BSTs are growth linked biodegradation tests, meaning the chemicals are usually added at a concentration that would result in growth and increase in bacterial biomass. Using a flow cytometer with a plate

reader, bacterial number can be measured for a large set of samples. To allow for high throughput and automation, incubation of environmental inoculum with test chemicals were performed in deep well plates with 1.2 ml sample per well. However, this small volume might make the already stringent BST even more rigorous as the number of bacteria, exposed to the chemical, would be even lower than in standard BSTs. Increasing the cell concentration in the wells are not an option, as there seems to be a saturation limit for growth, meaning that at high cell densities, biodegradation of chemicals is used for respiration only, not growth of biomass. To compensate for low bacterial abundance in the system, methods for enhancing the activity of the microbial community was tested: yeast extract was either added as a co-substrate at a concentration 1 to 100 times lower than test chemical concentration or added to inoculum and incubated for one week to adopt the inoculum to laboratory conditions before adding test chemicals. A set of eight reference compounds were included in the test method development: two readily biodegradable substances aniline and sodium benzoate used in standard BST, four biodegradable compounds with variable biodegradation rates (4-nitrophenol, oxipurinol, trimethoprim, benzotriazol), and two non-degradable compounds (acesulfame and sucralose). Four different inoculum sources were tested: activated sludge from a WWTP, water from an urban river, seawater, and water from laboratory cultures of *Daphnia magna*. Results from this experiment will be presented.

8.04.P-Th230 Cell- and Mathematical-Based Model Describing Synergistic Effects of Aromatic Hydrocarbons and Azoles on the CYP1A Biomarker

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Up to 140,000 chemicals are currently on the market in Europe and USA and many of these end up in the aquatic environment. Alternative in silico models are needed since it is impossible to experimentally test each potential chemical combination. Mathematical models can help us predict potentially harmful chemical mixtures while minimizing the number of required experiments. Induction of the aryl hydrocarbon receptor (AhR)-cytochrome P450 1A (CYP1A) signaling pathway, which mediates xenobiotic elimination, is commonly used as a biomarker for aromatic hydrocarbon exposure. However, AhR-CYP1A signaling can be affected by other chemicals, such as azoles. Azoles are a broad class of anthropogenic chemicals used as pharmaceuticals and pesticides, which are found in measurable quantities in the aquatic environment. Azoles are also known to inhibit vertebrate CYP enzymes, including CYP1A. We hypothesize that modulation of the AhR-CYP1A pathway by one chemical can affect the metabolism of another chemical that is modified by the pathway, by altering its biological half-life. We have developed a mathematical toxicokinetic model based on experimental data from a fish cell line (PLHC-1) that describes CYP1A and cellular chemical concentrations over time upon exposure to mixtures of the aromatic hydrocarbon, benzo(a)pyrene (BaP) and the azole, clotrimazole (CLO). We have exposed PLHC-1 cells to various combinations of these two chemicals and measured cellular chemical concentrations via GC-MS, CYP1A mRNA expression via qPCR, and CYP1A enzymatic activity via EROD assay at several time points. As expected, BaP and CLO mixtures result in synergistic (more than additive) and prolonged induction of CYP1A mRNA and enzyme activity. Based upon the known signaling pathway we developed a mathematical system of 10 ordinary differential equations to model AhR-mediated induction of CYP1A expression and cellular chemical concentrations. A portion of our experimental data was used to fit and parametrize the model while additional data not used in parametrization was used to validate the model. The model successfully describes the observed synergistic effects in CYP1A expression and enzymatic activity induced by combination CLO and BaP exposure and models cellular chemical concentrations over time. Mathematical models that describe cellular mixture toxicity mechanisms can help better predict mixture toxicity while minimizing the number of needed animal experiments.

8.04.P-Th231 Characterization of Non-Combustion Particulate Emissions From Rail Transport

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Transport-related emissions have been in focus of environmental protection for many years. For a holistic assessment of transport-related particulate emissions all modes of transport have to be considered. Rail transport is known as one of the most environmentally friendly transport systems. However, in addition to combustion-related emissions, abrasion from daily operation as well as infrastructure construction and maintenance measures represent a source of particulate matter. Studies regarding the different source contributions, particle size distribution, particle composition, and morphology of particulate emissions from rail transport have been scarce, making it difficult to assess the hazard potential. Therefore, further investigations are intended to close the gaps of knowledge in the field of non-combustion particulate emissions from rail transport. In a first project, the emission of particulate matter during infrastructure construction and maintenance work was investigated by means of aerosol spectrometry. Additionally, a part of the particulate matter samples was investigated by SEM-EDX to obtain information on chemical composition and morphology of the particles. Mineral components of gravel, as well as parts of elements relevant to railroads, e.g. aluminium, iron and copper from abrasion processes were identified. Therefore, current investigations within the framework of the BMDV Network of Experts, a research program financed by the German Federal Ministry for Digital and Transport (BMDV), focus on emissions from wheel-rail, contact wire and brake abrasion. Particulate matter at different test benches and in the field will be analyzed regarding its particle size, mass distribution and chemical composition. Based on the collected data, dispersion calculations will be carried out and consequences for the environment are highlighted. Moreover, a study on abrasion induced emissions will provide information on the particulate morphology (SEM-EDX) and its fate in the environment. Based on the

overall results, an initial risk assessment can be made, which will allow a significant contribution to closing the knowledge gaps and to gain a holistic understanding of particulate emissions in rail transport.

8.04.P-Th232 Characterizing the Effects of Chronic Conventional Heavy Crude Oil Exposure on the Growth, Development, and Behaviour of Larval Wood Frog (*Lithobates sylvaticus*)

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Much like oil transportation pipelines, amphibians are distributed worldwide, spanning a variety of ecosystems. Despite an identified need for additional information, few studies have examined the effects of oil spills on the early life stages of this group. Beginning in 2019, two parallel oil spill studies have been conducted at the International Institute for Sustainable Development Experimental Lakes Area in northwestern Ontario, Canada. The Freshwater Oil Spill Remediation Study (FOReSt) used in-lake enclosures to determine the effectiveness of non-invasive oil spill remediation techniques in different shoreline habitats and the Floating Wetland Treatments to Enhance Remediation (FLoWTER) study examined the efficacy of symbiotic plant-microbe relationships for *in situ* degradation of oil-derived hydrocarbons. In 2021, the effects of *in situ* chronic exposure to conventional heavy crude oil (CHV) (FOReSt study) or a CHV water-accommodated fraction (FLoWTER study) on the biochemistry, growth, development, and behaviour wood frog (*Lithobates sylvaticus*) tadpoles were characterized. Developmental and morphometric measurements were combined with the assessment of bioaccumulation and proteomic techniques to determine the effects of CHV exposure on the tadpoles. Bioaccumulation was quantified using a gas chromatography/tandem mass spectrometer to analyze total polycyclic aromatic compounds in whole body tadpole tissues. Another objective of the FLoWTER project was to characterize the influence of engineered floating wetland (EFW) presence/ absence on wood frog tadpoles. Within the FLoWTER project, weekly behavioural assays were applied to a subset of tadpoles to examine sociality, activity, and predator avoidance behaviours when compared to positive and negative assays. Results from the listed endpoints will be presented. Preliminary findings suggest the presence of a treatment effect on mean Gosner stage in the FLoWTER project. Effects on apical endpoints of oil treatments compared to un-oiled treatments in the FOReSt project will be discussed. This research will provide much needed data on the toxicological effects of CHV on amphibians which can then be used to inform risk assessment related to the over-land transportation of crude oil and potential impacts of freshwater oil spills on amphibians.

8.04.P-Th233 Chemical Strategy for Sustainability and Crop Protection Products: Untangling Interlinks Across the EU Regulatory Landscape

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European Union's chemicals regulatory framework is widely acknowledged as one of the most advanced systems in the world, ensuring high level of protection to human health and the environment. It encompasses numerous interdependent regulations covering a broad range of compounds and uses, such as industrial chemicals, plant protection products, biocides, chemicals in food and feed, fertilisers, medicinal and veterinary products, and cosmetics. However, the interlinks between different pieces of legislation may not always be given sufficient consideration – especially in a quickly-evolving regulatory landscape shaped by initiatives balancing state-of-the-art science with bold political vision. One such initiative is European Commission's "Chemicals Strategy for Sustainability" (CSS), intended to "boost innovation for safe and sustainable chemicals, and increase protection of human health and the environment against hazardous chemicals". The CSS publication kicked off a process of unprecedented ambition as it calls for revision of several pieces of legislation beyond core regulation, the launch of numerous non-regulatory initiatives and the engagement of a broad range of stakeholders, and that in a relatively short timeframe. During the discussions taking place throughout 2021 in various expert and political fora it became evident that the overhaul envisioned by the CSS was primarily focused on chemicals managed under REACH Regulation. Chemicals such as plant protection products (PPP) were not given sufficient attention, as the potential impact on sectorial legislation was not properly understood. This work presents an analysis of regulatory interlinks between different pieces of EU legislation, focusing on PPPs. We will show how changes proposed for REACH and CLP regulations (among others) can impact not just the approval of plant protection products in the EU, but also challenge the very foundation of risk-based approaches imbedded into PPP Regulation.

8.04.P-Th234 Chlordecone Uptake in Grasses: Relationship to the Anatomy and Physiology

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Chlordecone (CLD) is an organochlorine insecticide used to struggle against banana weevil (*Cosmopolites sordidus*) in French West Indies between 1973 and 1992. It is a long-term contaminant of soils and aquatic environment, classified as POP since 2009. Now, it pollutes banana watershed from banana fields to surface and marine waters. Plants growing in contaminated soils lead to human exposure by food, particularly by vegetable consumption. For livestock, the contamination through consumption forage grass-based, for a part. The accumulation of CLD in plants is a passive mechanism. It is highly variable and must depend on their physio-anatomical characteristics. Currently, no remediation method is or can be used on the huge polluted areas. So, the use of plants with efficient CLD extraction capacity could be a process of interest. Here, we had investigated which anatomical or physiological factors are favorable to the CLD uptake and accumulation in plants. Radiolabeled [¹⁴C]-CLD was used to study the CLD uptake and translocation to grass shoots growth in hydroponic conditions. CLD concentrations in wheat shoots were 5 higher than maize ones. As wheat had a poorer water use efficiency than maize (2.9 and 5.7 g.l⁻¹), it efficiently accumulated CLD in ratio of its biomass. In contrast, *Miscanthus giganteus*, with a WUE (6. g.l⁻¹), similar to that of maize, accumulated 6 times less CLD. The apparent TSCF (ratio of CLD absorbed to transpired water volume) is 14 times higher for wheat than for *M. giganteus*. This indicated various retention mechanism in CLD uptake according to plants. In addition to the presence of a rhizome, as in

Miscanthus spe, the anatomy of the roots contributed to this retention. Thus, CLD uptake in wheat roots was easier than in maize roots. The transfer rate through the rhizoderme was 5 times higher in wheat roots (6.8 vs. 1.3 ng/mm².h for maize). In contrast, the accumulated amount was twice as high in these last. That seemed correlate with higher dry matter and lipid contents in maize roots. The higher number of cell layers in these last (8 or 9 in maize and 3 in wheat) contributed to reduced CLD flux. In conclusion, the wheat CLD uptake was more efficient because both its higher rate of transpiration and a weak CLD root retention. These differences were of great interest to select plants for an efficient CLD remediation management. That was also useful to the monitoring of the chemical quality or forage for livestock risk assessment.

8.04.P-Th235 Chronic Effects of Venlafaxine and Its Main Metabolite O-Desmethylvenlafaxine on the Mollusk *Hydrobia ulvae*

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Venlafaxine (VEN) is a widely used antidepressant with 2643 kg prescribed in Denmark in 2020 alone. Venlafaxine and its main metabolite O-desmethylvenlafaxine (ODV) is released to the environment through the wastewater treatment plants, and recent studies have shown its worldwide distribution in river waters. Little is known about the ecotoxicological effects of VEN and ODV. The study aimed at assessing the environmental impact of sediment-associated VEN and ODV on a common mudsnail, *Hydrobia ulvae*, by examining important physiological and behavioral modalities related to behavioral effects. The deposit-feeding mollusk *H. ulvae* is widely distributed and abundant in Danish fjords and coastal waters. Three experimental setups were used to investigate the chronic effects of sediment-associated VEN and ODV on *H. ulvae*, using sediment concentrations of 0 (i.e., control) 0,01, 1 and 100 µg VEN or ODV /L. We examined: 1) The ability of *H. ulvae* to detect and avoid VEN and ODV contaminated sediment using two groups of snails; taken directly from the laboratory culture or pre-exposed to VEN or ODV for 26 days. 2) Feeding rate of *H. ulvae* before and after a 26 day exposure to VEN or ODV. 3) Growth rate of juvenile *H. ulvae* during a 26 day sediment exposure period to VEN or ODV. The results will be discussed.

8.04.P-Th236 Contamination of Food Through Microplastic From Plastic Cutting Boards

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Plastic cutting boards have been found to be the source of microplastic (MP) contamination in different foods, including beef, goat meat, and chicken as well as fish. In the cutting process of these foods, the boards emit small plastic pieces that either attach to the surface of the food or embed in the food itself. In this research, cut food samples were acquired from butchers, a fish market, and a supermarket chain in the Middle East. The samples were digested in 9.1 w% aq. KOH at 75°C for 10h. The cooled samples were filtered. The MP obtained was weighed and subjected to optical microscopy and infrared spectroscopy. The quantity and size distribution of the particles was determined using Fiji ImageJ software. Differential scanning calorimetry was carried out to understand the thermal behavior of the particles. The mean size of the MP in the raw meat (goat and beef) was found to be 1279.2 ± 835.0 µm. The maximum amount of MP observed in any of the foods was 1.64 ± 0.46 particles/g (goat) meat. Extensive washing of the meat reduced the MP count, but in all cases, residual contamination remained (min. 0.07 ± 0.05 particles/g). Chicken meat and fish contained fewer MP. Washing of the cutting boards with a high-pressure water stream after each cutting process reduces the MP count, but does not ensure that the next cut sample is free of MP. IR spectroscopy showed the MPs to be polythene. Meat containing MP was cooked in a pressure cooker, where MPs in the meat melted and recrystallized. Spent plastic cutting boards were acquired from butchers. From the 3D photo of a typical spent cutting board, it was calculated that 875 g polythene was emitted by the end of its lifetime, where 400 kg of goat meat has been cut. This would mean that one finds 2.2 mg plastic per g of cut goat meat so that on average 50% of the plastic will wash into the drain and either be retained in wastewater treatment plants or will be dispersed in the environment. This makes plastic cutting boards in the Middle East markets another significant contributor to MP in wastewater in certain regions. In addition, plastic cutting boards could be a source of MP in the environment.

8.04.P-Th237 Crude Oil Identification Using Linear Discriminatory Analysis (LDA) of Bicyclic Sesquiterpane (Bicyclane) Profiles in the Adipose Tissue of Exposed Fish

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Bicyclic sesquiterpanes (bicyclanes) are ubiquitous in crude oils and refined petroleum products, and upon exposure are sequestered in the adipose tissue of exposed fish. In this proof-of-concept study, we demonstrate the application of a linear discriminatory analysis (LDA) model to identify a source oil based on bicyclane biomarkers in adipose tissue extracts of exposed fish. In a laboratory trial, *Lates calcarifer* (barramundi, or Asian sea-bass) were exposed *via* diet (1% w/w) to a heavy fuel oil (n=9) or to Montara (n=9), a medium crude oil from the Australian Northwest Shelf, examples of which have both previously been spilled in the marine environment. An LDA model was then trained using a reference dataset of bicyclane fingerprint ratios of both exposure oils, plus four other NW Shelf crudes, two fuel oils, and eight weathered asphaltites from the Great Australian Bight for comparison. The LDA model correctly identified the respective exposure oils from a test dataset of corresponding bicyclane profiles in adipose tissue extracts of each of the 18 fish fed oil-enriched diets. This work demonstrates the potential of using adipose tissue bicyclane profiles in oil-exposed fish as a forensic identification tool for impact assessment and litigation purposes in an environmental oil spill, of particular use when the source of the spill is unclear.

8.04.P-Th238 Determining the Application of Small Extracellular Vesicles As Biomarkers of Arsenic Induced Urothelial Injury and Carcinogenesis Using a Label Free Quantitative Proteomics Approach

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Arsenic is a pervasive metalloid that continues to contaminate the water and food sources of millions of people globally. Among the numerous health effects of exposure are urothelial toxicity and potentially cancer. In recent years, small extracellular vesicles (SEVs) have been shown to be vital in intracellular communication and have been used in clinical studies as biomarkers of disease. Understanding the ways in which cells communicate during exposure is imperative and can allow for the detection of minimally invasive biomarkers of toxic responses. This study aims to investigate the SEV response of urothelial cells when exposed to arsenic, including the use of label-free quantitative proteomics to identify the key proteins in SEVs that are involved in early toxic response signalling. SVHUC1 human urothelial cells were exposed to 1, 2 or 5 μM sodium arsenite for 48 hours. SEVs in the culture media were isolated by differential centrifugation and were prepared for mass spectrometry using an optimized filter assisted sample preparation approach. SEV size ranged from 86-130 nm and the total SEVs released were significantly increased in transitional cell carcinoma. SEV origin was confirmed by the positive antibody array identification of several endosomal proteins. A total of 1408 proteins were identified, of which 35 were differentially expressed in arsenic exposure groups and 74 in transitional cell carcinoma. Pathway analysis revealed that those proteins differentially expressed in arsenic exposure groups were responsible for increased cell survival, migration, and invasion and decreased cancer cell death. Similar trends in pathway analysis of arsenic exposed cells and transitional cell carcinoma SEVs suggests potential links between urothelial toxicity and carcinogenesis. Further proteomics of paired cell lysate samples are being analyzed to determine if those proteins differentially expressed in SEVs are comparable to intracellular protein expression. This analysis will help to further elucidate the pathways responsible for arsenic induced urothelial injury and carcinogenesis while identifying novel non-invasive biomarkers of exposure.

8.04.P-Th239 Developing a Comparative Neurotoxicology Understanding of the Chiral Cyanotoxin Anatoxin-a in Two Common Fish Models

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Harmful algal bloom (HAB) toxins present risks to public health and the environment. The cyanotoxin anatoxin-a (antx-a) is a potent neurotoxin observed at the global level. To understand aquatic hazards of anatoxins, we initially conducted meta-analyses using probabilistic hazard assessments, observed diverse exceedances of guideline values, and identified aquatic toxicity data to be lacking, especially in terms of toxin purity, verification of treatment levels, and enantiomer specific and sublethal responses. To help address these gaps, we studied larval fathead minnow and zebrafish behavioral and molecular responses (gene expression, transcriptomics, and proteomics) following exposure to either (\pm) or (+) antx-a at environmentally relevant, analytically verified treatment levels, which were informed by probabilistic exposure distributions. We found duration and distance of fathead minnows swimming at the highest speed was significantly lowered by (\pm) antx-a in all but the lowest treatment level, while the opposite occurred with zebrafish, in which the toxin increased distance, duration, and changes in movement at the highest speed, though not significant. We examined gene expression focusing on genes related to neurotoxicity, oxidative stress, DNA damage, and hepatotoxicity. While there was little change in zebrafish, expression changes related to neurotoxicity and oxidative stress were identified in fathead minnows. This suggests fathead minnows may be more sensitive to this toxin based on these endpoints. We then studied purified (+) antx-a, the only enantiomer naturally produced, and observed high mortality in fathead minnows $>500 \mu\text{g/L}$ and largely refractory, lowered movement in zebrafish for most behavioral endpoints. These observations corroborate our sensitivity findings with (\pm) antx-a and highlights the increased toxicity of the purified enantiomer. We are currently performing gene expression analysis on these samples with an expanded profile based on tandem proteomics experiments. We further aim to develop full data independent proteomics and transcriptomic profiles using 3rd gen nanopore sequencing. Understanding of cyanotoxin effects in aquatic species under environmentally relevant conditions is necessary to support robust assessments and management of algal blooms, including improved risk predictions for HAB events.

8.04.P-Th240 Developing a Persistence Assessment Tool (PAT) to Support Consistency, Transparency and Robustness in Persistence Assessments

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Chemical persistence plays a key role in the determination of environmental exposure making it an important component in risk assessment and regulation. Interest in chemical persistence has increased significantly, highlighted by concerns from per- and polyfluoroalkyl substances (PFAS), (micro)plastic pollution and persistent, mobile and toxic (PMT) substances. Persistence assessment in the regulatory context involves comparing chemical degradation half-lives to set criteria for different environmental compartments (water, sediment and soil). Other information is also relevant for assessments (e.g. screening tests, non-standard experiments, QSARs, field data, etc), and should be considered following a 'weight-of-evidence' (WoE) approach. Implementation challenges remain in persistence assessments, particularly relating to guidance around the evaluation of data quality, and the WoE determination. There are also issues for substances whose properties render them difficult to evaluate using standard methods. This project, sponsored by Concawe, Cosmetics Europe and Cefic-LRI, and launched in February 2022, aims

to develop a Persistence Assessment Tool (PAT) to support the evaluation of persistence under regulatory frameworks such as EU REACH. This software tool will provide clear guidance and structure to evaluate data quality, and a quantitative WoE (qWoE) methodology to process the information input and calculate persistence conclusions. It will be applicable to all substance types and provide specific features for difficult and complex substances. The qWoE methodology will include some flexibility to allow users to adapt assessments to specific regulatory frameworks and other purposes. A further feature will be to incorporate a multimedia fate model to enable the determination of overall persistence. This will allow for potentially important additional environmental fate processes controlling persistence to be taken into account. The aim of the PAT is to provide support to practitioners responsible for undertaking persistence assessments, and to facilitate improved consistency, transparency and robustness in these assessments. During the development of the tool, we are keen to engage with the stakeholder community to seek their input and to maximise engagement. This presentation will provide an overview of the tool under development, the challenges it seeks to address, and information on how interested stakeholders can participate in its development.

8.04.P-Th241 Development of New Biobased Nanocomposites for Food Packaging Applications

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In this study, we investigate the properties of nanocomposites based on biodegradable PBSA which is food contact grade (complying to EU10/2011). We adopt the approach of mixing LDH fillers into PBSA to improve the overall durability and gas barrier properties of the nanocomposites. The commercially available SORBACID® 911 is our choice of filler due to its low-cost and also because it is listed as an acceptable material in the “Plastic Food Contact Materials” report (Regulation (EU) 10/2011). Moreover, considering that SORBACID® 911 is used on industrial scale as an acid scavenger for PVC materials it pre-sents an appropriate scalability for food packaging applications. The properties of PBSA-LDH based nanocomposites were investigated by rheological, tensile testing, thermogravimetric and optical methods, which demonstrate that the PBSA-LDH combination form compatible polymer composites with good dispersion. The addition of SORBACID® 911 fillers enhanced the gas barrier properties, in particular, the water vapour barrier, rendering the nanocomposites competitors to conventional plastic films used for food packaging [1]. Furthermore, the photo-durability of the PBSA-LDH nanocomposites is examined through accelerated photoaging and compared to natural weathering. The results demonstrate improved resistance to photodegradation and weathering compared to pristine PBSA, and the resistance towards both photoaging and weathering increases with the LDH concentration in the PBSA matrix. With the improved gas barrier properties together with increased durability of PBSA with added SORBACID® 911 we demonstrate a realistic opportunity for transitioning from non-biodegradable to biodegradable food-packaging.

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8.04.P-Th242 Development of Pull-Down Assays for More Targeted, Non-Target in Highly Complex Environmental Samples

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The global inventory of identified chemicals is increasing exponentially with databases including millions of natural and synthetic compounds. Chemicals that provide beneficial properties are synthesised industrially and exposure may become ubiquitous. A major challenge in protecting health and the environment is determining which compounds in use and emerging may prove to be a risk.

Effect direct analysis (EDA) first developed over 40 years ago is a tool capable of identifying the level of effect a complex mixture may have for a biological response, but it alone cannot pinpoint all compounds of effect. The development of affordable ultra-high resolution mass spectrometers over the last decade has paved the way for omics style detection but there is a trade off between identifying as much information as possible in a sample and detecting the compounds of relevance. Pull down assays may provide the tool required to filter complex mixtures to specific endpoints.

A pull-down assay uses a binding protein that normally binds specific endogenous compounds but in exposure to xenobiotics that mimic endogenous compounds can potentially also bind these compounds. Here we present the work for development of pull-down assays for Retinoic acid receptor alpha protein (RAR- α).

Proteins were synthesised in house and the extraction protocol optimised through use of known binding compounds followed by the application of the method using biological environmental samples. Extracts were ran on LC-Orbitrap-Fusion in both positive and negative with multiple positive and negative controls applied for quality control and assurance. Processing was performed using multiple free and vendor software's for cross comparison.

With complex bio-assays the pull down was capable of taking a mixture of many thousand features and through application of negative and positive controls the data was reduced to only those compounds that were bound to the protein.

This method opens the way for a more targeted approach to non-target with only the compounds of interest being pulled out from complex matrices. There will still be a need for additional compound detection and identification, but the overall data is far more manageable. The work was supported by the Czech Science Foundation Grant No. 20-04676X, and MSCA 839243.

8.04.P-Th243 Development of Relevance and Reliability Criteria for Hydrocarbon Degradation Half-Lives in Soil and Sediment

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Under European environmental regulations (REACH, PPP, BPR), a PBT/vPvB assessment is required for most chemicals, taking account of the relevant constituents and transformation/degradation products. Persistence (P/vP) assessment relies on compartment specific degradation half-lives (HL), which must be compared to the criteria laid down under annex XIII of REACH. Definitive biodegradation HL data are typically derived from high tiered OECD degradation simulation tests in surface water, sediment and soil. These tests are technically complex and not applicable to petroleum UVCBs, since the test methods are most appropriate for mono-constituent substances. Difficulties in attaining such demanding P/vP assessment obligations have been recognised by regulators and some flexibility in the P/vP assessment framework exists. For example, according to REACH Annex XIII, a weight-of-evidence (WoE) approach can be used to bring in other non-standard test data to form conclusions on P/vP. Recently, efforts to improve the robustness and transparency in WoE for regulatory decision making have been described, however clear guidance is lacking regarding the assignment of reliability and relevance of data which displays naturally high variability in degradation half-lives. This can lead to useful degradation data being discarded for regulatory purposes because of a lack of certainty on how to assess their relevance. In this work, relevance and reliability criteria were developed based on non-standard test method similarity to OECD 307, OECD 308 test guidelines and concordance with ECHA guidance. A simplified scoring system of “high”, “medium” and “low” was used to assess the relevance and reliability of individual data from a database of non-standard experimental half-lives for hydrocarbons in soil and sediments covering 952 and 1243 datapoints, respectively. This approach permits data to be ‘weighted’ accordingly when drawing conclusions during P/vP assessment. These relevance and reliability criteria may inform testing entities on methods to improve the quality of the testing outcome. It is also useful for curating data to be used in predictive biodegradation models. The database, along with relevance and reliability criteria will be presented. This work is applicable to European regulations and may be extended to other relevant pieces of global legislation.

8.04.P-Th244 Ecotoxicological Effects of Sugarcane Vinasse on the Tropical Insect *Chironomus sancticarloi*

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Vinasse is the main liquid residue generated in the ethanol and sugar production's process. Vinasse is rich in organic matter and nutrients, with acidic pH between 4.0 and 4.5 [1]. The main destination of this residue is the fertirrigation of the sugarcane fields, which is fundamental for reducing the consumption of water and synthetic fertilizers. However, due to the huge volume generated (each liter of ethanol produces 13 L of vinasse), excessive and long-term fertirrigation can cause environmental problems. Anaerobic digestion is one of the most attractive methods for vinasse biodigestion, integrating low operating costs with the possibility of obtaining commercial added value sub-products and energy recovery as biogas in a combined system (acidogenic + methanogenic reactors) [2]. In this context, this study evaluate the effects on the survival of *Chironomus sancticarloi* exposed to vinasse *in natura* (influent) and biodigested vinasse in methanogenic reactor (effluent). The liquid matrix used in the ecotoxicology tests were the influent and the effluent of a lab-scale (2.1L) methanogenic anaerobic structured bed reactor (AnSTBR) treating sugarcane vinasse. The tests were carried out using the methodology adapted from the OECD guideline 235 [3]. Samples of vinasse (Effluent) and vinasse *in natura* (Influent) were diluted in 6 different concentrations: 0.1, 0.4, 1.56, 6.25, 25, 100% (Short-term test) and 5 different concentrations: 0.6, 0.9, 1.35, 2 and 3% (long-term test). For the short-term exposure, the Two-Way ANOVA [4] showed that the dilutions 0.1 and 0.4% were statistically similar to the control (p-value > 0.05) for both matrix. Also, the effluent 1.56% showed to be more toxic than the influent 1.56%, being statistically similar to the effluent 100% (p-value of 0.7792). Regarding the long-term test, only the interaction between the 2% dilution and the influent showed statistical different compared to the control (p-value of 0.008). The results showed that the methanogenic treatment slightly decrease the mortality rate in the short-term and long-term exposure (EC50 of 1.25% (v:v) compared to 1.66% (v:v) and EC50 of compared 1.11% (v:v) to 1,26% (v:v), respectively). However, in both exposure time vinasse showed to be extremely toxic for the *C. sancticarloi* and the reuse of the vinasse showed to be a good alternative to avoid the incorrect discharge in water bodies.

8.04.P-Th245 Effects of Diesel Exhaust Particles on the Health and Survival of the Buff-Tailed Bumblebee *Bombus terrestris* After Acute and Chronic Oral Exposure

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Biodiversity loss is one of the main concerns in the twenty-first century. Over the last forty years approximately 60 % of the populations of mammal, bird, reptile, and amphibian species went extinct. Environmental pollution is one important driver of this loss. Anthropogenic activity, especially the diesel-powered transportation sector, is a major producer of environmental pollution in the form of micro- and nanoscale diesel exhaust particles (DEP). In addition to DEP, incomplete combustion processes produce polycyclic aromatic hydrocarbons (PAH). These PAHs are the assumed cause of the particles' toxicity. Such particle-based pollution accounts for a substantial part of respiratory diseases in mammals. However, despite of this awareness, we only know

very little of the effects of DEP and PAHs on invertebrates. A large number of wild plants are pollinated by insects, some even predominantly or exclusively by bumblebees. Close to areas with heavy traffic highly active, flying insect pollinators are likely exposed to high amounts of DEP and thus might suffer potential negative effects. Those negative effects could trigger an even greater loss of plant diversity, which pose nutrition, hiding spaces etc. to a lot of organisms. We exposed individuals of the buff-tailed bumblebee *Bombus terrestris* to different concentrations of DEP/ sugar water solution suspensions in acute and chronic oral exposure experiments to investigate dose- dependent effects of DEP on survival and fat body content, as a proxy for an insects' health condition. Acute exposure to DEP showed no effect on survival or fat body content of *B. terrestris*. However, chronic exposure to high doses of DEP lead to a significantly reduced survival of bumblebees by almost 50 % compared to controls. Further, the fat body content of DEP exposed bumblebees was significantly reduced in bumblebees exposed to 0.5 g/l DEP compared to controls. Next to the dietary exposure we investigated the effects of DEP on bumblebees via airstream. For that, we exposed the bumblebees to an airstream with defined particle concentrations and performed electroantennography and scanning electron microscopy after exposure. There was no significant reduction in amplitude intensity in bumblebees exposed to DEP compared to the control. Our results give insights into how accumulation of high concentrations of DEP e.g., near heavily trafficked sites, can influence insect pollinators' health and survival.

8.04.P-Th246 Effects of Eprinomectin on the Reproduction of Earthworms and Springtails *Raphael Souza*¹, *Cleiton Souza*², *Ana Paula Braga*³ and *José Roberto Guimarães*⁴, (1)University of Campinas UNICAMP, Brazil, (2)ASR Analytical & Scientific Research, Brazil, (3)São Paulo State University UNESP, Brazil, (4)UNICAMP University of Campinas, Brazil

Eprinomectin (EPR) is a veterinary drug belonging to the avermectin family and it is commonly used to combat a wide variety of parasites. Large quantities of veterinary drugs are used by rural producers in order to maintain the high productivity of the livestock sector and reduce financial losses caused by different diseases, but they can cause an environmental impact. Approximately 90% of EPR can be excreted in the dung of treated animals and can be found in soil in concentrations ranging from ng/g to ?g/g. So, the objective of this study was analyzing the effects of different concentrations of EPR in a tropical soil on the reproduction of earthworms (*Eisenia Andrei*) and springtails (*Folsomia candida*). For this purpose, soil was contaminated one day prior the experiments with the following concentrations of EPR: C1 = 0.5 ?g/g; C2 = 1.0 ?g/g; C3 = 2.0 ?g/g; C4 = 4.0 ?g/g; C5 = 8.0 ?g/g; C6 = 16.0 ?g/g; C7 = 32.0 ?g/g; C8 = 64.0 ?g/g; and C9 = 128.0 ?g/g (C9 was not carried out in the experiment with earthworms). As the stock solution used to contaminate the soil was prepared with ethanol, a treatment containing soil+ethanol was also performed. A negative control containing uncontaminated soil was also carried out. Experiments were based on ISO 11267 and ISO 17512-1. Ten adult specimens of springtails were placed in glass containers with 30 g of soil for 28 days and then the number of juveniles was counted; ten adult specimens of earthworms were placed in glass containers with 500 g of soil for 28 days and the counting of the number of juveniles occurred 56 days after the beginning of the experiment. According to the statistical analysis, reproduction of springtails was significantly reduced in all concentrations. The exposure of earthworms to the contaminated soil revealed significant decrease in the number of juveniles in concentrations C1, C2, C3, C6, C7 and C8. Decrease in the number of juvenile springtails can be related to the high mortality rate of adults during the exposure, indicating high toxicity to the species. Decrease in the number of juvenile earthworms has been found to other avermectin drugs, such as abamectin, by other researchers in both tropical and temperate climates. So, the results suggest that EPR can affect the reproduction of these two species of soil invertebrates. Financial Support: FAPESP (17/26214-8)

8.04.P-Th247 Effects of Microplastic on the Population Dynamics of a Marine Copepod

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Microplastic is ubiquitously and persistently present in the marine environment, but knowledge of its population-level effects is limited. In this study, to quantify the potential theoretical population effect of microplastic a two-step approach was followed. First, the impact of microplastic (polyethylene, 0.995 g cm⁻³, diameter 10-45 µm) on the filtration rate of the pelagic copepod *Temora longicornis* was investigated under laboratory conditions. It was found that the filtration rate decreased at increasing microplastic concentrations and followed a concentration-response relationship, but at microplastic concentrations below 100 particles L⁻¹ the filtration rate was not affected. From the concentration-response relationship between the microplastic concentrations and the individual filtration rate a median effect concentration of the individual filtration rate (48h-EC₅₀) of 1956 ± 311 particles L⁻¹ was found. In a second step, the dynamics of a *T. longicornis* population were simulated for realistic environmental conditions, and the effects of microplastics on the population density equilibrium were assessed. The empirical filtration rate data were incorporated in an individual-based model implementation of the dynamic energy budget theory to deduct potential theoretical population level effects. The yearly averaged concentration at which the population equilibrium density would decrease by 50% was 593 ± 376 particles L⁻¹. The theoretical effect concentrations at population level were a fourfold lower than effect concentrations at individual level. However, the theoretical effect concentrations at population level remain three to five orders of magnitude higher than ambient microplastic concentrations. Since the present experiment was short-term laboratory-based and the results were only indirectly validated with field data, the in situ implications of microplastic pollution for zooplankton field populations remains to be further investigated.

8.04.P-Th248 Effects of Plastic on the Freshwater Snail *Biomphalaria Glabrata*

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Since the 1950s, plastic has become increasingly important as a material. However, its long life and decomposition into microplastics cause significant environmental pollution. Therefore, students of the Master's programme "Ecotoxicology and

Environmental Management” investigated the effects of conventional (high density polyethylene) and biodegradable plastic bags (based on corn starch) on the reproductive behaviour of the freshwater snail *Biomphalaria glabrata* within one year starting from an open-ended problem-based learning (PBL) case. The application of the 7-step PBL-method allowed the students to design their experiments independently under guidance and with feedback from the lecturers. For the experimental set-up, 6 snails each were placed in jars with tap water, lettuce leaves and cut pieces (approx. 3 x 3 cm, 1 g) of a conventional or biodegradable plastic bag. The treatments were tested in duplicate. The exposure experiments lasted 6 weeks and the reproduction of the snails was observed weekly by their laying behaviour. To test the effect of substances that can leach from plastic bags, pieces of plastic were exposed to tap water for a week before the snails were incubated in the leachate. A new leachate was prepared each week. In addition, the surface of the two types of plastic was examined with a scanning electron microscope (SEM). The exposure experiments showed that *B. glabrata* reacts to the added plastic pieces with increased clutch laying. In particular, snails exposed to conventional plastic reacted with a significant increase in clutches. It was noticeable that the egg clutches were mainly laid on the plastic pieces. However, snails incubated in the plastic bag leachate (without plastic pieces) did not show an increased egg laying behaviour compared to the negative control. The SEM images showed that the conventional plastic bag had a smoother surface than the biodegradable bag. The study showed that the reproduction of the *B. glabrata* is influenced by plastic but it seems that the increased clutch production is not due to leached substances. It is possible that the altered reproductive behaviour in snails exposed to plastic is related to other circumstances such as the surface area and amount of plastic. However, further experiments are needed to better understand the effects of plastic on snails. Financial support from the City of Vienna project PBL in Molecular Life Science (21-06) is gratefully acknowledged.

8.04.P-Th249 Environmental Fate of Venlafaxine and Its Main Metabolite O-Desmethylvenlafaxine

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Venlafaxine is a widely used antidepressant with 2643 kg prescribed in Denmark in 2020 alone. Most wastewater treatment plants (WWTP) are not equipped to remove pharmaceutical compounds, which results in the continuous distribution of active pharmaceutical ingredients to the aquatic environment, including venlafaxine (VEN) and its main metabolite O-desmethylvenlafaxine (ODV). Little is known about the environmental concentrations and fate of VEN and ODV when released into the environment. We conducted: 1) An environmental screening study of the influent-, effluent water and sludge from the local WWTP (Bjergmarken, Roskilde) in addition to water- and sediment samples collected at selected locations from the recipient estuarine ecosystem, Roskilde fjord, Denmark. 2) A study investigating the distribution of VEN and ODV in a laboratory setting, using a water-exposure/sediment system. VEN and ODV was added to the surface water to obtain a concentration of 2 mg/L. The concentrations in water and sediment compartments were sampled continuously during a 28-day period to follow the distribution kinetics between water and sediment compartments. The samples were analyzed with Solid Phase Extraction and QuEChERS extraction procedure followed by gas chromatography coupled with mass spectrometry. 3) An investigation of the environmental fate of VEN and ODV in sediment, under the influence of the sediment dwelling *Hydrobia Ulvae*. This served to elaborate on the complexity of the fate of pharmaceutical compounds within the aquatic environment.

8.04.P-Th250 Environmental Pollutant Monitoring Along Railway Tracks in Germany

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In general, rail transport has a lower release of pollutants in direct comparison with other modes of transport. However, over 180 years of rail operation on today's more than 33,000 km of tracks in Germany have an impact on the environment. In this context, inorganic and organic substance inputs from today's and past rail operations and associated infrastructure maintenance works, for example the vegetation control, treatment of wood sleepers, are of particular importance. The study aims at the characterization of the spectrum of released pollutants and their dispersion in the track and the surrounding environment. The obtained results are important to close knowledge gaps and provide a basis for a comprehensive environmental risk assessment. Five long-term monitoring sites have been established to identify pollutants and characterize their transport behaviour. The investigation program includes the analysis of i. a. heavy metals, herbicides (incl. metabolites), and PAHs in seepage water, ground- and surface water as well as in soil samples. As part of the monitoring site characterizations, the depth-dependent herbicide contamination of all substances used in rail transport since 1985 was determined in different soil horizons and track areas. In addition, the degradation potential in the track and edge areas was also investigated. All sampling and analyses were carried out using standardized methods. The results show a site-specific pollutant distribution, with a clear decrease in pollutants from the centre of the track to its vicinity. Especially after vegetation control, the currently applied herbicides glyphosate, flumioxazine, and flazasulfuron can be detected in the soil and in the leachate. Within the scope of degradation tests, a degradation of the currently applied herbicides was proven at all sites. In addition, other herbicides such as atrazine, bromacil, DCMU (diuron), and ethidimuron were determined in the soil at specific sites. In a direct comparison between in- and outflowing groundwater in the track area, arsenic, iron, and zinc were found, with none of the values exceeding the regulated limits. The study is the first step in a series of investigations aimed at gaining a comprehensive understanding of the potential pollutant emissions in the track area. These studies will contribute significantly to a sustainable development of the transport system.

8.04.P-Th251 European Freshwater Silver Monitoring Data Do Not Suggest a Potential European-Wide Risk

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European legislations frequently focus on substances that are of potential concern to human or environmental health, like ‘priority substances’ under the Water Framework Directive 2000/60/EC (‘WFD’) that are identified as substances posing a significant risk to or via the aquatic environment. To properly assess the potential risk of a substance, high-quality representative monitoring data should be compared to a safe threshold concentration. In this work, several publicly available monitoring data sets for silver in the freshwater environment are assessed and checked for a potential European-wide risk according to the methodology used by the European Commission. Most of the available silver monitoring data sets contain a large proportion of undetected samples with a reported concentration below the limit of quantification (LOQ) of the analytical technique, leading to considerable uncertainty in the data set. For silver, this LOQ is often at or above the safe threshold concentration and the way undetected samples are treated during the data processing considerably impacts the final conclusion. We demonstrate that for large data sets covering many European countries (and often a wide range of LOQs), the uncertainty in the data set does not allow to make any general conclusions about European-wide risk for silver. However, by examining the data sets in more detail and examining three additional country specific monitoring data sets, we show that silver does not pose a risk to the freshwater environment. We conclude that the available data sets need careful assessment to account for the values that are below the LOQ, and that there is currently no reliable evidence indicating a European-wide risk for silver in the aquatic environment, meaning it should not be selected as priority substance under the WFD.

8.04.P-Th252 Evaluating Brittleness and Fragmentation of Conventional Polymers and Biopolymers Upon Photoaging

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SEALIVE (Strategies of circular Economy and Advanced bio-based solutions to keep our Lands and seas alIVE from plastics contamination) is a H2020 innovation project, which vision is to reduce plastic waste and contamination on land and in seas by boosting the use of biomaterials and contributing to the circular economy with cohesive bio-plastic strategies. As part of WP7, polymer degradation is investigated in several real service life and end-of-life conditions, in order to assess the life cycle of polymers and to eventually develop international biodegradation and ecotoxicity standards for bioplastics. Particularly, urgent concerns of polymers’ end-of-life are focused on the potential hazards and risks associated with microplastics. A prevailing source of microplastics is the fragmentation of larger plastics or product wear, however studies on the rates of fragmentation of polymers under various conditions are scarce. Photodegradation of polymers leads to structural changes of the polymer backbone, such as oxidation with formation of carbonyl functional groups, chain scission and cross-linking. These transformations at molecular level impact the overall mechanical properties of the plastic and favour embrittlement and fragmentation, leading to the generation of micro- and nano-plastics. Understanding the fragmentation rates and behaviour of photo-aged macroplastic generating secondary microplastics will provide vital information to enable a full risk-assessment of plastics needed for international biodegradation and ecotoxicity standards. Here we present a comparative study between a photoaged conventional polymer, polyethylene (PE), and an emerging biopolymer, polylactic acid (PLA), with a focus on the interrelation between photoaging and brittleness. Changes in morphological and physico-chemical properties of the photoaged polymer films are followed through the use of melt rheology, DSC, FTIR, UV-VIS, SEM and durometry with the aim to identify most relevant polymer properties, such as molecular weight, crystallinity and tensile properties that could explain the brittleness and fragmentation behaviour of aged polymers. In this work, we demonstrate the relationship between the brittleness behaviour of the aged polymers with the crosslinking and chain scission mechanisms which occur during the photoaging. **Acknowledgments** *The work in this paper is supported by the SEALIVE Project which is funded by the European Union's Horizon 2020 Program, under grant agreement number 862910.*

8.04.P-Th253 Evaluation of Risk of Organophosphorous Flame Retardants in Drinking WATER Sources

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Flame retardants are functional additives used in many articles to reduce flammability and prevent fires from starting or spreading. They play an important role in improving the fire safety of articles used in industry and everyday life. Following the restriction and phase out of many polybrominated diphenyl ether (PBDEs) flame retardants because of their hazardous and persistent nature, the use of organophosphorous flame retardants (OPFRs) has increased, OPFRs are not chemically bound within the material and there will be release of OPFRs from articles into the environment through volatilisation, dissolution, and attrition. OPFRs are more water soluble and more mobile than PBDEs with greater potential for long-range transport via waterborne routes. They could be considered as persistent, mobile organic chemicals and may pose a risk to surface, and ground waters including those used as source waters for drinking water supply. There are many reports of the widespread occurrence of OPFRs in the environment, including remote regions far from likely, local sources. Studies have shown that OPFRs could have harmful impacts on human health including neurotoxicity, carcinogenesis, and endocrine disruption activity. Internationally, there is widespread recognition that the replacement of PBDEs with OPFRs may be a case of regrettable substitution. Regulatory authorities are responding with policies that gather data, inform consumers, and limit the use of flame retardants of concern. To understand whether OPFRs are likely to occur in source waters and drinking waters in England and Wales at levels that could present a risk to human health, a desk-based evidence review is being conducted. Evidence has been collated and synthesised to describe (i) which OPFRs are likely to be present in water used for drinking water supply (ii) the range of likely exposure concentrations in raw waters (groundwater and surface water sources) based on reports of occurrence (iii) known uses, sources and pathways of release

that would indicate potentially high sources/concentrations in a catchment and (iv) removal efficiencies of OPFRs through treatment for drinking water supply have been collated and synthesised.

8.04.P-Th254 Evolution of the Electricity Mix in the Italian Context

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“It depends on the energy mix” is a very common output in Life Cycle Assessment studies. However, the electricity datasets many studies rely on are not up to date. In a context like the European one, with mixes evolving rapidly due to energy transition and changing geopolitical context, an old dataset can significantly affect the results. Moreover, studies of cutting-edge technologies might need to consider the change in the energy systems at the time the technology will be available on the market. The current study focuses on the Italian electric system as a case study. An updated and detailed LCA of the electricity mix of the last four years in Italy has been performed. Data from the national Transmission System Operator and statistics from Eurostat along with environmental certificate of the main power plants have been used to perform a LCA of the current mix. The scenarios developed in the framework of the Integrated Climate and Energy Plan and of the Green Deal have been taken as the reference for the evolution of the electricity mix in Italy in 2030, and have been modelled to evaluate the effect of the transition on the mix. In both cases (current and future mixes) the consumption mix has been considered as the national production (without pumped hydro generation) plus the import minus the export. The allocation method cut off has been applied. Plants have been classified per technology and energy source. Each plant type is defined in terms of efficiency and load factors. For future mixes, the technological progress was taken into account through enhanced conversion efficiencies and load factors. Data from the ministry of the economic development, from the Transmission System Operators for Gas have been used to model the geographical origin of oil, gas and carbon, and data from the Energy Service Operator to recreate the mix of biogas and biofuels. The evolution of the electricity mix presents a continuous reduction in the climate change impact in the last four years. All the impact categories show a reduction in the scenarios in 2030 compared to the last current year, except for the resource consumption. A correlation between the level of decarbonization and the increase in resource consumption is detected. If “it depends on the energy mix”, it is important to have an updated and detailed mix available for LCA studies. Knowing in advance the trend of the impacts of our future mixes will help determine the direction of almost every LCA study.

8.04.P-Th255 Experimental Arena Size Influences Larval Zebrafish Photolocomotor Behaviors and Response Thresholds Following Exposure to the Model Neurostimulant Caffeine

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Behavioral experiments with fish and other species are useful, sensitive tools for studying a wide variety of contaminants. However, many behavioral endpoints can also be highly variable, so understanding how experimental parameters influence detection of behavioral changes, including responses to environmental stressors, represents a key issue in comparability and applications of these experimental designs within and among species. Previously multiple sizes of experimental arenas have been used to measure photolocomotor changes with age/size and for diverse endpoints under investigation. However, there remains a need to understand whether behavioral responses are influenced by experimental arena size and if they differ after exposure to a contaminant. Thus, we initially defined baseline swimming profiles of larval zebrafish under light and dark conditions using a ViewPoint ZebraBox across different plate sizes (96, 48, 24, 12, and 6 well). We then performed studies with the common neurostimulant caffeine (0.003 – 112 mg/L) and zebrafish, and analytically verified caffeine treatment levels using liquid chromatography – tandem mass spectrometry. Following caffeine exposure, we observed behavioral responses (i.e. distance travelled, number of discrete movements, and duration of movements at different speed thresholds) using these different observation areas. We found that swimming total distance of naïve fish increased logarithmically with increasing size of the observation area while standard deviation initially increased and then decreased with increasing plate well size. Under light conditions, significant behavioral responses to caffeine were not observed in the smallest wells, but were then more pronounced with larger arena size. Similarly, we observed significant stimulatory responses, including bursting duration, count number and distance traveled, only in the largest arena size, but these effects were not identified in smaller wells. Our results indicate that increasing well size leads to higher movement levels, and performing larval zebrafish larval behavioral assays in smaller observation arenas may not detect or underestimate photolocomotor response thresholds to chemical contaminants. Understanding these changes can improve comparability among experimental designs with different arena sizes and demonstrates the importance of characterizing potentially confounding variables during behavioral assays.

8.04.P-Th256 Exploring Biological Read Across of Endocrine-Mediated Effects Between Birds and Other Vertebrates

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Regulation 1107/2009 states that for approval, pesticide active substances require a consideration of endocrine disrupting (ED) properties for humans and non-target organisms. Currently, environmental safety testing for endocrine properties include tests in mammals, fish and amphibians (OECD, 2018). The available test for avian species is limited to the one generation avian reproduction study (OECD TG 206), which includes only apical endpoints. The US EPA’s avian 2-generation toxicity test in Japanese quail has not been validated by the OECD as it was considered unresponsive for EATS (estrogen, androgen, thyroid and steroidogenesis) modalities. In addition, it is unclear from available guidance whether these studies would be sufficient to draw a conclusion on ED potential in birds. As there are various anatomical and hormonal differences between birds and other vertebrates

(e.g. different body temperatures, the production of cleidoic eggs, production of uric acid to void nitrogenous waste, different sexual differentiation mechanism) it is necessary to understand how responses of birds to endocrine active chemicals compare to better studied vertebrates. There is also the regulatory requirement to avoid unnecessary animal testing. Here we review the evidence for biological extrapolation of endocrine-mediated effects between birds and other vertebrates for which agreed test methods exist (mammals, amphibians and fish). We consider the usefulness of existing methodologies/study guidelines and guidance in the identification and evaluation of potential endocrine-mediated effects in birds. We also briefly compare the hormone systems and ADME (absorption, distribution, metabolism and excretion) processes of birds and other vertebrates. Through a review of published *in vivo* case studies with known ED chemicals targeting the EATS modalities, we compare observed effects in birds with those in other vertebrates. We discuss the question of whether potential ED chemicals for birds are likely to be captured by existing tests on non-avian vertebrates. Our assessment suggests there is a good basis for biological read across between birds and other vertebrate taxa, and also provides some suggestions for future research.

8.04.P-Th257 Exposure to Arsenic From Drinking Groundwater and Methylation Efficiency on the Redox Status of Blood Tissue in Populations of the Colombian Caribbean

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Inorganic arsenic (InAs) is considered to be the principal form of arsenic (As) in groundwater. US Environmental protection Agency guideline for arsenic in water is 10 µg/L. Lifetime average daily dose (LADD) is considered an important method for exposure assessment, because the effects of toxicity of InAs may be accelerated with an increase in exposure dosage. Exposure to arsenic leads to increased production of reactive oxygen species (ROS) and other toxic intermediates through its biotransformation, which can subsequently cause oxidative stress and alterations of the cellular system. However, these effects can vary largely among individuals, possibly due to demographic, anthropometric factors, smoking history, lifestyle and genetic factors, increasing the susceptibility to As toxicity. Therefore, this study evaluated the risk of As exposure in humans and the possible effects of the average daily dose and methylation efficiency on the redox status in blood tissue in people exposed from drinking groundwater. Specific surveys on 155 individuals aged between 18 and 81 years old were applied used to collect demographic and anthropometric information of the subjects and their lifestyle. The groundwater and urine samples were analyzed for assessing total As, using HPLC-HG-AFS. Likewise, the exposure and risk of As was assessed by LADD method and the Hazard quotient (HQ) and the methylation efficiency was determined for urinary speciation. Besides, the redox status of blood tissue was determined by enzymatic oxidative stress tests such as Superoxide dismutase (SOD) and Catalase (CAT), as well as with enzymatic oxidative stress tests such as total Glutathione (GSH) status, using the OxiSelect™ kit from Cell Biolabs. In addition, to determine the superoxide ion, the cytochrome C reduction method was used. The effects of the average daily dose of arsenic on the redox status in blood tissue were tested using a multivariate analysis, adjusted by potential confounders. Sixteen groundwater wells from studied municipalities were analyzed. The study population was divided post hoc into two subgroups: a subgroup exposed to arsenic above optimal levels (n=55), with aquifer concentrations of 16 µg/L, and a subgroup exposed to arsenic below optimal levels, with aquifer concentrations of 7.2 µg/L (n=100). The As exposure dose in the subgroup with high exposure was of 0.31 µg/kg-bw/day, generating a risk HQ=1.1. The urinary arsenic species concentrations were 0.80 µg/L for InAs, 0.60 µg/L for MMA^V and 1.2 µg/L for DMA^V. The values of the enzymatic oxidative stress tests: SOD and CAT were higher in individuals from the subgroup with high arsenic exposure (p= 0.03 and p= 0.04 respectively). Besides, were positively correlated with urinary total As metabolite concentrations. Exposure to inorganic As was associated with increased oxidative stress among individuals exposed to arsenic from groundwater. Therefore, SOD and CAT could serve as biomarkers for assessing the effects of As on redox status.

8.04.P-Th258 Foamed Plastic on Shorelines - a Study of Sources and Polymer Composition of Litter From Danish Beaches

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Foamed plastic contributes importantly to the environmental pollution globally, however, knowledge of the types, sources and composition of this litter pool is limited. The aim of this study was to characterize item types and polymer materials of foamed litter from six Danish reference beaches during 2018-2021. A total of 1962 foamed items were registered during the study, corresponding to 12% of all plastic litter at the beaches. The foamed litter were classified into ten categories, including specific identifiable item types, as well as foamed PS fragments, or pieces of rigid or flexible sponges consisting of other foamed materials. Litter of foamed PS could be recognized with large accuracy by visual assessment, however, only a relatively small proportion of the foamed PS litter were identifiable items such as cups and food containers for take-away, packaging materials or buoys and floats. Hence, PS fragments constituted 31% of the total number of foamed litter and hence, a better source identification of this unidentifiable fraction may be critical to establish effective regulatory measures to reduce littering. Foamed litter of any other types than PS were visually classified into two categories of insulation/wrapping or foam sponges, consisting of rigid foam and flexible foam, respectively. The majority was assumed to origin from land-based sources, e.g. construction and packaging. FTIR spectroscopy was used to determine the polymer distribution of the foamed litter, and PS (44%) and PUR (49%) were identified as the major foamed polymers. In contrast, other polymers such as PE, PP, PVC, PA and EVA were only found in relatively small numbers. PUR comprised 84% of all the rigid foam or flexible sponges. Principal Component Analysis of FTIR spectra of PUR items demonstrated distinct clusters with specific spectra features that were typical for rigid and flexible PUR foams. These differences were associated to chemical fingerprints of the NCO/OH ratio, which are related to the hard-segments (diisocyanates) and soft-segments (polyols) mixed to produce the PUR foam. Finally, we also investigated the use of different

reference spectral libraries for accurate polymer assignment of the PUR litter based on FTIR data and correlation methods. The analysis supported that reference libraries covering several variants of PUR including weathered PUR are important.

8.04.P-Th259 Fractionation and Identification of Target Alkyl Phenols and Naphthenic Acids in Produce Water Discharge
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Produced water (PW) is the water co-produced with oil and gas from oil wells and it represents the largest volume waste stream in oil and gas production operations on most offshore platforms. In 2021 there was an estimated 30 million tons of produced water discharged from the Danish continental shelf alone. Current regulation monitor only the dispersed hydrocarbon content on the legal limit of 30 mg/L; this limit does not address the polar species dissolved in the water which are potentially the more environmentally concerning constituents in produced water e.g. Naphthenic acids and Alkyl Phenols. Naphthenic acid comprises a complex mixture of aliphatic, cycloaliphatic and aromatic carboxylic acids that naturally occurs in the oil fraction and from added production chemicals. They act as surfactants and have shown to be toxic to a variety of organisms, especially in aquatic environments. Alkylphenols naturally occurs in crude oil, but they also came from degradation products of alkylphenol ethoxylates (APEOs, a group of non-ionic surfactants) and from the degradation of other production chemicals. Nonylphenols (NP) and Octylphenols (OP) are included in the European Union's list of priority hazardous substances for surface waters. The aim of the study was to isolate fractions of phenols and NAs from samples of produced water from the Danish oil production wells using solid phase extraction. The extracts were measured for target analysis with LC-ESI-MS and the total acidic content of the NAs fraction was determined by Fourier-transform infrared spectroscopy, as a fast and low-cost analysis for identifying functional groups and quantification. Finally, the toxicity of the extracted fractions was assessed using the metabolic activity of bioluminescent bacteria (*Aliivibrio fischeri*) and growth inhibition of algae (*Skeletonema* sp.).

8.04.P-Th260 Gas Chromatography x Cyclic Ion Mobility-Mass Spectrometry: A Novel Platform for the Comprehensive Separation and Discovery of Unknown Per- and Polyfluoroalkyl Substances

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Per-/polyfluoroalkyl substances (PFASs) have been widely used since the 1940s in industry and everyday household products. They also persist in the environment and bioaccumulate in humans and wildlife. Although PFASs number in the thousands, very few, e.g. perfluorooctanoic acid and perfluoro-octanesulfonic acid (PFOA and PFOS), are subject to regulations aimed at protecting the environment and health. The number of PFASs introduced to the global market has rapidly outpaced research on their environmental impact, and the identities of up to 90% of PFASs in environmental and biological samples are unknown. Gas chromatography hyphenated with cyclic ion mobility (GCxIMS) is a two-dimensional separation technique that promises to accelerate the discovery of unknown PFAS. It can resolve molecules that differ by shape, which can be approximated as a collision cross section (CCS). The cyclic geometry of the mobility cell enables ions to travel successive passes, increasing their arrival time and relative separation. In this contribution, we explore the potential for this novel technique to tackle complex environmental samples and identify unknown persistent organic pollutants (POPs). By modelling the CCS of approximately 20,000 chemical compounds produced in or imported into Canada, we show that most POPs, including PFASs, are characterized by high molecular density, having a relatively compact CCS relative to other industrial chemicals of similar mass. When this filter is applied to GCxIMS data collected from a set of indoor dust samples, POPs, including per- and polyfluoroalkyl substances (PFAS) are revealed. Validation of this approach was performed using a standard reference material (SRM 2585) of indoor dust. We show for the first time that mixed halogenated n-alkanes, ubiquitously used as lubricants and flame retardants, are contaminants of indoor air. The method is also applied to samples collected from industrial emissions in Ontario, Canada.

8.04.P-Th261 Greenhouse Gas Emissions and Sea Level Rise Impact on Coastal Ecosystem Services Within the LCIA Framework

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While providing essential ecosystem services (ES) to people such as food and raw material provision, touristic and cultural opportunities, as well as climate regulation or infrastructures protection, coastal ecosystems are among the most affected natural systems by anthropogenic activities. Greenhouse gas (GHG) emissions lead to temperature increase and – among other effects - to sea level rise (SLR). This poses significant threats to coastal environments; SLR will alter the distribution of coastal assets and habitats and will result in changes in the services they provide to people. ES are barely assessed in life cycle assessment (LCA). To date, the only efforts to model ES impact pathways in the Life Cycle Impact Assessment (LCIA) framework have focused on few terrestrial ES linked to land use such as erosion control, biotic production or water filtration. Potential impact of climate change, acidification or eutrophication – among others - on ES are therefore occulted in LCA impacts profiles, leading to a possible underestimation of the results. This project proposes to address this gap by developing characterization models and factors expressing the potential cost to society resulting from the change of coastal ES supply triggered by climate change and SLR. We firstly propose a novative approach to assess SLR impacts in LCIA by developing characterization factors that assess changes in coastal habitats due to GHG emissions. To this end, we use a recent digital elevation model combined with SLR projections based on different emissions scenarios and a global habitat mapping. This cartography of flooded habitats is then combined with a

mapping table linking habitat type and ecosystem services. Finally, well-known ES monetary valuation tables are used to assess the economic value of the ES supply changes

8.04.P-Th262 Guideline Values for Per- and Polyfluoroalkyl Substances (PFAS) in Soils - a Survey at EU Member State Level

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Per- and polyfluoroalkyl substances (PFAS) are counting some thousands of chemicals, all of them containing at least one fully fluorinated carbon atom. PFAS have been manufactured for decades for a broad range of technical uses in industrial processes and products, resulting in intentional or non-intentional environmental release. PFAS are highly persistent against abiotic and biotic degradation, and diffuse contamination of even remote areas across the globe by geochemical transport is well-known. Further, accumulation in biota and subsequent food-chain transfer of some PFAS is causing concern regarding long-term adverse effects on human health and wildlife. However, only for a limited number of PFAS extensive toxicological and ecotoxicological studies have been conducted so far, as it is the case for the prominent perfluorooctanoic acid (PFOA) and perfluorooctanoic sulfonic acid (PFOS). Consequently, only a few PFAS have been regulated within the EU or via the Stockholm convention until today. Global diffuse contamination as well as local point contaminations known from a lot of sites across the EU will remain a long-term threat. Contaminations stretching over several kilometres are usually located near the facilities of fluorochemical industry or sites where PFAS are used in production processes or PFAS-containing waste was disposed-off. Soil and groundwater are often severely contaminated in these areas, while smaller scale pollution is typically caused by the use of firefighting foams containing PFAS. In both cases, local authorities have to decide how to deal with the damage, e.g. what remediation or safeguarding measures to take, what land uses are still possible and whether the excavated soil can be reused. Since there are no scientifically derived regulatory values for PFAS-contaminated soils effective at EU level, respective national values have been derived by several Member States. To establish the scientific basis for PFAS soil guideline values under the German Soil Law, we started to gather and review publicly available respective values from several EU member states. The presentation will provide an overview of the results of this exercise. Similarities and differences between national values will be highlighted – from both a regulatory and scientific point of view.

8.04.P-Th263 How Safe Is Nanomaterials to Brain?

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Although brain is protected by a tight physiological guardian named blood brain barrier (BBB), deposition of engineered nanomaterials (ENMs) in brain and consequent neurotoxicity has been reported. To date, it is still unclear whether and how ENMs enter the brain by crossing the BBB. Understanding the potential of ENMs to cross the BBB as a function of their physicochemical properties and subsequent behavior, fate, and adverse effect beyond that point is vital for evaluating the neurological effects arising from their unintentional entry into the brain, which is yet to be fully explored. This is not only due to the complex nature of the brain but also the existing analytical limitations for characterization and quantification of NMs in the complex brain environment. Herein, we conducted an interdisciplinary study by using a novel analytical workflow and *in vitro* BBB model, as a complex biological barrier, to determine and quantify the biotransformation of metallic NMs as a function of their physicochemical properties and correlate the influence of the biotransformation to the BBB-penetration ability and transport pathways. We found metallic ENMs transform in the BBB as affected by their shape, size and intrinsic solubility, which in turn modulates their transport form, efficiency and pathways through the BBB, and consequently their neurotoxicity. Very little was transcytosed to the basolateral (brain) side of the BBB model, with significant amounts being recycled back to the apical (bloodstream) side and limited retention in the BBB cells. Paracellular transport was only observed at the higher concentration tested and was associated with membrane damage and NM dissolution. The generated data about biotransformation modulated uptake and transport of NMs through BBB open a new horizon for medical application of NMs, e.g. targetable drug delivery systems for brain diseases and also for biological fate assessment of NMs in brain to support their risk assessment.

8.04.P-Th264 How Useful Can Non-Invasive Sperm Collection in Amphibians Be for In Vitro Toxicology Testing?

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The decline of Amphibian populations constitutes a critical concern in conservation biology and pollution has been identified as a major driver for such declines. Limited information on the toxicity of chemicals to amphibians exist and most of the available standard toxicity assays for amphibians involve *in vivo* experimentation, which makes difficult their risk assessment considering the increasing amount of chemicals to be tested and the ethical issues involved. An urgent need to develop non-animal assay alternatives for amphibians is warranted, however the replacement of animal testing by *in vitro* methods requires the validation of suitable cell models, preferably obtained using non-lethal/invasive methods. Besides somatic cell-lines, gamete cells may also be considered as an alternative to animal experimentation in risk assessment. In amphibians, such methodology can be very relevant since most anuran amphibian species release gametes directly into the water column for external fertilization, hence both eggs and sperm enter in direct contact with water contaminants. This confers a high ecological relevancy to *in vitro* assays using sperm cells, since effects observed may be related with reproductive success. However, few works have been published on the effects of chemicals on amphibian's sperm cells (25,26), and most involve the sacrifice of males to remove the testes and obtain sperm cells

as testicular macerates or suspensions from sexually mature males. However, anuran spermatozoa can also be collected through hormonal induction, either in spermic urine through abdominal massage or in cloacal fluid through cannulation of the cloaca. This communication presents a review compiling relevant data and describing current knowledge on the use of non-lethal sperm collection in anuran amphibians species and discussing its potential use in the development of in vitro toxicity testing.

8.04.P-Th265 Implementation of a Dual Ionization Source, ESI and APCI, for Determination of Organic Contaminants in Drinking WATER by LC-MS/MS

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The water directives of the European Union define quality standards of water for human consumption and set forth measures to monitor priority substances that are deemed high risk to humans (1). However, the large number of organic contaminants defined in the chemical parameters to be monitored in water set an analytical challenge. Indeed, the molecules belong to very different chemical compound classes and a reliable separation and sensitive determination by LC-MS/MS would require to use of many different methods separation and detection methods. Here we present our developments of analytical methods to a sensitive determination of organic contaminants in drinking water using a QSight LC-MS/MS system with dual ion source. In particular, we explored the capability of a dual ionisation mode, ESI and APCI, to improve method sensitivity. Initial developments on acrylamide showed a reduction of ion suppression without comprising of sensitivity when using APCI versus ESI ionization. Still permitting a method quantitation limit for acrylamide of 0.01 µg/L. Subsequently, using the compounds chlorate, perchlorate and N,N-dimethylsulfamide (DMS) we implemented a method where the mass spectrometer switches the acquisition between APCI and ESI within the same LC-MS/MS run. Here, first eluting DMS was measured using APCI source, and then the mass spectrometer switches to ESI source to measure both chlorate and perchlorate. The method shows a sensitivity of 0.01 µg/L for all compounds which are well below the parametric values required by EU regulation. References: 1. DIRECTIVE (EU) 2020/2184, available: <https://eur-lex.europa.eu/eli/dir/2020/2184/oj>

8.04.P-Th266 Improved Environmental Impact Assessment of High Salinity Produced Water

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When crude oil is produced large amounts of water are produced along with the organic petroleum material. This co-produced water (PW) is highly saline and contaminated from contact with crude oil in the formation and the production additives, thus it is heterogeneous and remarkably complex. Considering that this water is being discharged to the sea at most current offshore production sites, reaching the ambition of zero harmful discharge requires stringent produced water management and improved monitoring and toxicology evaluation. This work aims at an initial characterization for improved environmental impact assessment of water production in connection with offshore oil, facing the analytical challenges given by a hypersaline and carbon-rich water sample. This work presents a workflow for complex aqueous samples from oil wells starting with total alkalinity (TA) analysis of samples purified with respect to organic dissolved content and on the analysis of major inorganic anions and specific volatile and semi-volatile organic acids content using liquid ion chromatography (HPIC). The determination of trace elements (As, Cr, Cd, Co, Cu, As, Sb) in produced water has also been targeted using ICP-OES for analyzing non-digested, hot plate digested and microwave digested samples and comparing the results. Matrix matching for blanks, calibration standards and quality checks have been implemented to improve plasma stability, measurements reproducibility and raw data reliability. The same trace elements have been targeted in suspended particulate matter (SPM) of PW via cellulose 0,45 µm filters and using acidic digestion. In addition, due to the complexity of the matrix, for various trace elements, SPE pre-concentration techniques have been also applied and verified in order to minimize the matrix influence on analytical detection. Further characterizations on suspended particulate matter in PW samples have subsequently been carried out through scanning electron microscopy. This presentation includes the initial results of these analyses of actual Danish North Sea produced water as well as discharge point water samples.

8.04.P-Th267 Improving Polymer Identification of Micro- and Mesoplastic Particles Ingested by Seabirds - Potential for Adjustment of Biomolecule Interferences in Fourier-Transform Infrared (FTIR) Spectroscopy

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Pre-processing of FTIR spectral data can often be beneficial for the identification of the polymer-based constituents in plastic materials, e.g. by applying baseline correction and smoothing. In some cases, correction of spectral information for matrix effects can also improve the quality for identifying the polymers. In this case, 427 and 227 plastic particles > 1mm ingested by the storm bird Northern fulmar (*Fulmarus glacialis*) from West Greenland and North East Greenland, respectively, was analysed using a diamond ATR-FTIR spectroscopy. Even after wash and drying of particles, many of the spectra did still contain clear signals from also protein-like biomolecules in addition to their typical polymer spectra, because these biomolecules had been sorbed to the plastic. This biomolecule interference had therefore in many cases effects on both the Hit Quality Index (HQI) values and the specific polymer assignments when using spectral reference libraries for identifying the polymers. Manipulating the spectra by adjusting for this matrix effect could therefore improve the confidence in polymer identifications by reducing risks for false positives and false negatives. Other outcomes can be to improve systematic quality criteria for positive polymer assignments and to be able to handle spectral data for many samples more homogeneously in batch analyses and not only assess them one by one. Following steps of pre-processing were included in this study: Smoothing, baseline correction, normalisation, and in the end subtraction with a spectrum previously isolated from gut-fluid exposed plastics in fulmars for reducing the influence of matrix, because of the potential interfering biological protein-like signal. The results of these data treatments showed that 18 % re-assignments of the polymer ID when applying HQI values >0.7 for library matches and a significant increase in HQI-values >0.9

for especially particles consisting of PE (polyethylene) and PP (polypropylene). Only analysing raw un-processed FTIR spectra increased thereby the risk for false polymer assignments. In addition, comparison of spectral reference libraries with or without spectra for important types of environmental weathered plastic materials showed that inclusion of weathered plastic materials in reference libraries also can improve the confidence of polymer assignments of ingested plastic particles from fulmars.

8.04.P-Th268 In Silico and Experimental Screening Platform for Characterizing Environmental Impact of Industry Development in the Arctic - an Overview of the Project EXPECT

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The Arctic environment is facing evident changes such as temperature increase and consequent rapid decline in the sea ice. These conditions will favour the development of industrial activities, e.g., oil and gas exploration, tourism and aquaculture. In order to assess the potential environmental risks from the anticipated industrial development in the Arctic, methods and approaches that elucidate the pollution pathway from the emission to the environment to the display of adverse outcomes in the biota should be developed. EXPECT will develop *in silico*, *in vitro* and *in vivo* approaches for an integrated assessment of impacts of combined Arctic pollution on relevant marine organisms and populations. The experimental outputs will feed into the development of a Source-To-Outcome-Pathway (STOP) to characterise the causal source-exposure-effect-impact relationships for pollutants and their mixtures. The primary objective is to characterise the most relevant Arctic STOPS and use these to perform Cumulative Risk Assessment (CRA) to support sustainable management of existing and future industrial and anthropogenic activity in the Arctic. EXPECT is a 4 years project (started Q3/2021) that includes research groups from Norway (NIVA, Akvaplan-niva and SINTEF), UK (Newcastle University) and USA (Clemson University). Specific work packages include 1) the development of Aggregated Exposure Pathways (AEP) for characterisation of emission sources to external and internal exposure concentrations; 2) the development of Adverse Outcome Pathways (AOP) for the priority toxicity drivers, endpoints and susceptible taxa; 3) hazard assessments of single pollutants and different mixtures; 4) performance and experimental evaluation of a STOP-informed CRA. The outcomes from EXPECT are anticipated to support more mechanistically-informed environmental management, including optimal coastal zone planning and sustainable industrial development. EXPECT is funded by the Research Council of Norway (project # 315969) and supported by NIVA's Computational Toxicology Program (NCTP, www.niva.no/nctp).

8.04.P-Th269 Integrating Hydrothermal Liquefaction in Wastewater Treatment: Impact on Biological Nitrogen Removal

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Hydrothermal liquefaction (HTL) is a thermochemical conversion technology proposed to replace anaerobic digestion in wastewater treatment plants (WWTP). In order to achieve a net positive energy production as well as nutrient recovery by integrating HTL in WWTPs, investigations on how the wastewater from the HTL process (HTLWW) affect the microbial activity involved in the nitrogen cycle should be addressed. The lack of knowledge on how to treat HTLWW and how to integrate this effluent in the WWTP constitutes a significant obstacle to the industrial scale production of biocrude. In this context, this study investigated the inhibitory effect of HTLWW on the nitrification and denitrification processes and its potential as carbon source for the heterotrophic denitrifiers. In the Nitrification inhibition assays, three different concentrations of the HTLWW were tested 0.05, 0.15, and 0.3% v/v. In the denitrification assays, the inhibition of HTLWW was tested with 0.3% and 1% v/v HTLWW. Denitrification rates of HTLWW, acetate, glucose, starch, and settled influent wastewater from Fredericia WWTP were tested as carbon source. The average nitrification rates over 6 hours of incubation were 0.62 ($\pm 11\%$), 0.48 ($\pm 13\%$), 0.37 ($\pm 24\%$), and 0.19 ($\pm 33\%$) mgN-NH₄⁺·gVSS⁻¹·h⁻¹ for the blank, 0.05, 0.15, 0.3% v/v HTLWW treatments, respectively. The nitrification inhibition relative to the control showed a linear relationship with the HTLWW concentration from which a half-maximal effect concentration (EC₅₀) of 0.21% v/v was estimated. Even though the highest denitrification was obtained for the highest content of HTLWW, there was no significant difference (ANOVA test with $p > 0.05$) between the control and the 1% v/v treatments. The experiments using acetate, glucose and HTLWW yielded the highest nitrate reduction rates of 3.4-4.0 mgN-NO₃⁻·gVSS⁻¹·h⁻¹ and there no significant difference among them ($p > 0.05$). The highest nitrite accumulation was obtained when glucose was used as carbon source. The effect of HTL process water components on nitrification and denitrification have been little explored and our results confirm inhibitory effects that cannot be neglected as 50% nitrification inhibition may be expected when HTL is integrated at a typical WWTP. Relatively high levels (0.3% v/v) of HTLWW showed some (17%) denitrification inhibition, but at the same time proved to be a nearly as good carbon source for denitrification as acetate and glucose.

8.04.P-Th270 Intrinsic Properties of Metallic Contaminants Govern Bioaccumulation Behaviour: An Ex-Situ Comparison of Bulk Metallic, Nanomonometallic and Nanopolymetallic Bioaccumulation in Benthic Isopods

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The accumulation of metallic contaminants in benthic fauna is well documented, but less is known about the behaviour of emerging nanoscale contaminants. The likelihood of trophic transfer from benthic fauna to higher lifeforms makes elucidating these behaviours vital to our understanding of broader ecological risk and the ultimate fate of contaminants in the environment. This study examines the *ex-situ* uptake and accumulation of analogous bulk (macroscale) and nanoscale metal contaminants in benthic isopods, as well as comparing the accumulation of monometallic and polymetallic compounds. Results show that bulk and nanoscale contaminant accumulation exhibit qualitatively similar behaviour; however, accumulated nanoscale contaminant concentrations were an order of magnitude greater than those of the bulk analogues. This highlights the greater bioavailability of

nanoscale contaminants in the benthic environment. Monometallic and polymetallic compounds showed qualitatively and quantitatively similar accumulation behaviour, despite the inclusion of an additional metallic species in the latter contaminant. Of greater interest, however, is the relative uptake of different metallic species from the polymetallic compounds. The relative accumulation of each species was found to be imbalanced, indicating that the organism preferentially accumulated one ionic component of the compound over the other. Furthermore, this preference was found to correlate with the elemental thiophilicity of the ionic species, indicating a thiol-mediated mechanism of accumulation. These findings improve our understanding of the behaviour and fate of metallic contaminants in the benthic environment. The increased relative ecological risk of nanoscale contaminants is demonstrated, and the influence of thiophilicity on the bioavailability of contaminants is demonstrated at nanoscale for the first time.

8.04.P-Th271 Investigating the Effects of FFP2 Mask Leachates on *Eisenia fetida* and of Microplastic Beads on *Caenorhabditis elegans*

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The COVID-19 pandemic has caused an extensive increase of plastic waste. In 2020 over 1.5 million face masks have entered the environment, mostly due to improper disposal. Face masks consist almost entirely of plastic, mainly polypropylene, which is non-biodegradable. Still, over time, depending on weathering conditions, they are disintegrated into smaller particles, microplastic particles, which then can be taken up by soil organisms. Furthermore, plastic can be a vehicle of toxic substances, f.i. in the form of additives that have the potential of leaking out of the masks into the environment. To test the effects of additives in FFP2 masks on the earthworm *E. fetida*, avoidance tests were performed according to the ISO 11268-2 guideline. Adult *E. fetida* were exposed for 48 h to 450 g/L leachate of used FFP2 masks, which was obtained by column percolation according to DIN 19528. Then their avoidance behavior was investigated. As a positive control, 30 g/L sodium chloride was used as an alternative to the teratogenic boric acid. A significantly higher number of specimens was found in the leachate, compared to the negative control. Two FFP2 masks were subjected to a MOSH/MOAH analysis, once leached with n-hexane, once with dH₂O as solvent. This resulted in over 4000 mg/kg for MOSH in n-hexane, dH₂O showed values below 4 mg/kg. Furthermore, two transgenic strains of *C. elegans*, KWN190 (intestinal damage) and CL2166 (oxidative stress) were exposed to fluorescent microplastic beads for 96 h. Then, GFP signaling was investigated under a fluorescence microscope, and viability and brood size were investigated. An uptake of microplastic beads could be observed in all specimens. No clear difference of GFP signals between exposed and negative control was detected. On days 2 and 3, a slight increase in viability and reproduction could be observed. The results of the experiments indicate effects of microplastic and FFP2 mask leachate on the tested organisms. Even though the high MOSH results are of rather little meaning, as the solvent n-hexane does not represent a natural leaching process of masks, it must be noted, that there is a possibility of leaching organic substances out of the FFP2 masks via saliva and by touching the masks. However, further research needs to be done with larger sample sizes and different, more fitting solvents regarding the leachate and more sensitive test organisms.

8.04.P-Th272 Investigating the Impacts and Potential Mitigation Options of a Mixture Assessment Factor (MAF) for Substance Chemical Safety Assessments Under REACH

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Environmental risk assessments under EU regulations are generally conducted on a per substance basis. However, in the environment substances may occur in the presence of other substances, and this has led to concern from risks posed by unintentional mixtures of substances. A potential policy measure to mitigate this risk is to apply a generic assessment factor to existing risk assessments of substances to account for unintentional mixtures. This so-called mixture assessment factor (MAF) would therefore increase the conservatism of existing risk assessments in proportion to the magnitude of the MAF. Where a MAF is applied to existing risk assessments, this may lead to the risk assessments no longer demonstrating safe use (risk characterization ratio, RCR > 1). In these cases risk assessments will need to be revised. The MAF is currently proposed to be implemented under the EU REACH regulation for industrial chemicals, as part of the Chemicals Strategy for Sustainability. However, the precise details, such as at what magnitude the MAF will be set, are yet to be decided. In this study, a methodology has been developed for investigating the impacts of, and identifying possible mitigation measures for, a MAF for substances registered under REACH. This methodology has been applied to the environmental components of chemical safety reports (CSRs) of three substances, where a MAF of 10 has been applied. A stepwise approach has been developed, which first identifies those uses for which environmental RCRs are above 1 with the application of the MAF. For these impacted uses, possible mitigation options were explored, including (1) refinements to existing exposure assessment assumptions, (2) data generation, (3) increased risk management measures (RMM), and (4) reduction in amount used. The outcome of the investigations for the three example substances will be presented as case studies. The implications of MAF, further mitigation options, and potential opportunities to improve mixture risk assessment will be further discussed.

8.04.P-Th273 Issues in Cooperation of Science and Industry: How Make Better Use of Our Combined Know-How and Resources All the Way From Life-Cycle Science to Improved Life-Cycle Products and Services in Industry for a More Sustainable World

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There are two Spheres that still do not cooperate optimal: The Sphere Science and the Sphere Industry. The topic is not new. Walter Kloepffer wrote clear words about it in 2001 in „Two planets and one Journal“ and over 10 years later in 2012 22 experienced LCA experts from academia, industry and consulting did refresh the topic in the article „LCA’s theory and practice: Like ebony and ivory living in perfect harmony?“. However, we are still being far away from cooperating well, as we do not use our combined know-how and resources in a best possible way, but waste time to argue our different standpoints. We are losing time that we do not have to save our planet. Neither protectionism of industry nor arrogance of science will lead us forward. Society needs good science and good industry practice, products and services. Best societal benefit is made from good cooperation of both. There are good and bad examples of cooperation among the spheres: e.g. the SETAC Working groups in the 1990s, followed by the SETAC UNEP Life-Cycle-Initiative and the UNEP GLAD / GLAM Initiative and the Product Environmental Footprint as fundamental instrument to steer the European Green Deal. We will discuss in this presentation challenges, hurdles and mistakes, which we learned in the last 25 years and which still are partly being made today in the LCA cooperation of science and industry. We aim to inspire improved understanding and co-working within the different spheres. We will touch on concrete issues, evidence and aspects like data (collection), flow lists, regionalization, unit conversion, data set types, data import-export, data quality, data detail, fundamental different LCA system modelling, mixing of data sets and modelling approaches, documentation and last but not least on the important differentiation of “latest science” and “good practice”. Results and data in “LCA science” reflects a different aim, than “LCA results and data” in industry. Understanding this difference will lead to improved life-cycle cooperation as necessity of a more sustainable future.

8.04.P-Th274 Laboratory Survey on Assessing Vitellogenin in Fish

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The fish protein vitellogenin (VTG) is an important biomarker for possible endocrine activity of substances acting via oestrogen, androgen, or steroidogenesis pathways. There are, however, concerns regarding the assessment and reporting of this endpoint because it is highly variable as demonstrated by historical control analyses both within and amongst laboratories. Therefore, the possibility for equivocal, false positive and/or negative outcomes is high. Consequences of this may be the requirement for additional fish testing to confirm or refute changes in VTG, and a possibly erroneous identification of endocrine activity. With the aim to gather technical information and understand the reliability of VTG results, a survey was prepared in a collaboration between the UK’s NC3Rs and a working group of industry and consultancy experts. The survey covered a range of aspects including general methodologies for sampling, measuring and interpreting VTG concentrations in different fish species and matrices. The survey was sent to 27 laboratories in August 2021 and was completed by 16 (6 from the UK, 3 from the USA, and 7 from the EU). The main focus of these organisations was testing for regulatory purposes (using fathead minnow (*Pimephales promelas*), zebrafish (*Danio rerio*) or Japanese medaka (*Oryzias latipes*)) although two respondents measured VTG as part of field monitoring using a range of different fish species. The survey results confirmed variability in background VTG concentrations (using the internal historical control datasets of the surveyed labs) associated with different fish batches, or changes in fish husbandry or laboratory practices. These aspects were considered difficult to control. There were also differences related to the general methods for measuring VTG. With a focus on measurement of the VTG protein using enzyme-linked immunosorbent assay (ELISA; the most commonly used method for VTG analysis), differences amongst laboratories will be discussed covering: 1. Sample collection and storage, 2. VTG quantification, 3. Data handling, and 4. The benchmark for data acceptability. The survey highlighted some differences amongst laboratories, which have the potential to impact the VTG results. The survey results provide the basis for further discussion to improve the harmonisation of VTG measurements in fish and an opportunity to reassess the suitability of current acceptability criteria for VTG measurement.

8.04.P-Th275 Launching an Open-Source Global Chemical Inventory

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A pioneering effort to index the global chemical inventory, *i.e.*, all chemicals registered on (super-)national economic markets, was first published by Wang *et al.* in 2020.^[1] This ground-breaking work has enabled a greater understanding of the regulatory chemical space and allowed for widescale read across and analyses of the inventories and their chemicals. As part of the H2020 project ZeroPM (<https://zeropm.eu>), a fully reproducible and open-source re-construction of the global chemical inventory will be developed. Re-evaluating and updating previously included and several new inventories, a modern database infrastructure will be established using PostgreSQL as *lingua franca*. The databases will support singular, structurally defined chemicals, *i.e.*, by a unique InChI string, as well as advancing the integration of mixtures, UVCBs, polymers, and moieties. Using InChI strings allows for a robust identification of unique substances, and will allow to establish workflows for, *e.g.*, reproducible SMILES creation using Open Babel and large-scale scanning for potential moieties of interest across all included molecular structures. Wherever possible, cross-references will be made, *e.g.*, from a well-defined mixture to its individual constituents and to their moieties. Individual functionalities for the databases will be implemented within orchestrated Docker containers. The database itself will be downloadable in flat, tabular formats (*e.g.*, as CSV file), and eventually a graphical user interface will be provided for simple access to the database. In addition, application programming interface (API) functionality will be provided to programmatically access the database. The development of the databases will follow FAIR principles, and both the resulting data and code will be shared on the Zenodo community (<https://zenodo.org/communities/zeropm-h2020>) and the GitHub repository

(<https://github.com/ZeroPM-H2020>), respectively, under permissive licenses. The ZeroPM project and its databases will contribute to the European Commission's Chemicals Strategy for Sustainability, and will support open, transparent research projects benefitting both the environment and the general public. ^[1] Wang *et al.*, 2020. *Env. Sci. Technol.* **54**:2575–2584, doi:10.1021/acs.est.9b06379

8.04.P-Th276 Life Cycle Assessment As Affirmation Tool for Faster Uptake of Construction Products Incorporating Waste Materials

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According to Eurostat, the total production of cementitious materials in the EU was estimated to be 163 million tonnes in 2016, corresponding to an estimated turnover of €15.2 billion. Currently, the annual production of cement in Croatia is around 4.4 million tons. At the same time, the global average CO₂ emission per tonne of cement is estimated to be around 0.83 tonnes. Considering the expected rapid population growth and production increase, if no actions are taken by 2050, the production of cementitious materials alone could contribute to 24% of total global CO₂ emissions. There is no other construction material (natural or man-made) that is produced in such high quantity as concrete, therefore a phase-out strategy for cementitious binders in concrete is highly unlikely. In East & south-east Europe (ESEE) countries today, the main cementitious binders used are those with a significant amount of industrial by-products from heavy industry, such as slags from the steel industry and fly ash from coal combustion. However, for these industrial by-products to work, they must have a certain quality and stability in production quantities. Since these criteria cannot be met with regional industry, most industrial by-products are currently imported from countries such as Turkey or China. Such heavy reliance on the import of secondary raw materials poses risks: supply insecurity due to scarcity, regulations and/or geopolitical factors and price volatility of industrial by-products, depending on regulations and market opportunities. Transformation of the construction industry into low-environmental and resource-efficient ones is possible through greater circularity of the different types of waste streams that are still disposed of in landfills. This includes opening new value chains and industrial symbiosis, adapting and redesigning solutions, and creating a framework and standardisation for wider use of construction products incorporating waste materials. LCA presents an important and efficient tool in the affirmation process for faster uptake of these products. This research shows two different LCA examples of different local available wastes used as reactive binders in the construction sector and a comparison of their environmental impact with existing commercial cement. In addition, the greatest challenges and barriers (social and technical) of accepting new materials in the construction sector have been identified through interviews with the key stakeholders.

8.04.P-Th277 Life Cycle Assessment of Na-Ion Batteries With Ti1Al1TiC1.85 MXene As Negative Electrode: From Laboratory to Industrial Scale

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Energy storage can be the key for the fast decarbonization of the electricity grid and the transportation sector. Nowadays, Lithium-ion batteries (LIBs) are the most used battery chemistry, however, still show critical issues linked to their production, energy consumption, greenhouse gas emissions and rare raw materials use. Thus, it is important to find alternative storage technologies to be used singly or in combination with LIBs. Sodium-ion batteries (SIBs) are promising battery technology and possible successors to LIBs, with good theoretical environmental and electrochemical performance. Particularly, MXenes are promising materials for energy storage applications such as anodes for sodium-ion batteries. SIBs environmental impacts are essential: Life Cycle Assessment (LCA) is a consolidated methodology and is the main assessment approach followed in this study. The LCA is complemented with an economic indicator, the Commodity Life Cycle Costing (CLCC) which quantifies, in economic terms, a product's use of natural resources during its life cycle. When technologies are still under development, the LCA methodology application presents two main criticalities: the lack of data and the effective technology implementation and use in future contexts. It is, therefore, necessary to adopt an approach that allows both to evaluate the impacts of emerging technologies on an industrial scale and consider the system's future evolution. Hence an Ex-ante LCA, using LIBs as a proxy for the transition from laboratory SIBs to industrial scale, has been carried out. In this study, coin cells with Ti₁Al₁TiC_{1.85} MXene as anode material at three different technological grades: laboratory, pilot and industrial are assessed. LCA results allow to compare technological scales and to provide useful information to improve related processes with a view to eco-design. The assessed SIBs result less environmentally friendly than LIBs, an outcome driven by the lower energy density of the SIBs. The CLCC indicator calculated for SIBs batteries is more than seven times higher than that of LIBs, meaning a higher natural resource use or the use of more expensive/scarcer resources. However, if only critical materials are considered, SIBs are characterized by lower values than LIBs. Therefore, EU must encourage research for alternatives to LIBs in the next years, to fulfil its ambitious decarbonization goals.

8.04.P-Th278 Liquid Chromatographic Determination of Per- and Polyfluoroalkyl Substances in Environmental River WATER Samples

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Contaminants of emerging concern (CECs) such as per- and polyfluoroalkyl substances (PFAS) have attracted significant interest of researchers, policymakers, and water treatment facilities. This is because PFAS are highly persistent in the environment and tend to be bio-accumulative thus causing adverse effects to terrestrial and aquatic life. Therefore, there is a need for simpler and fast methods for the determination of PFAS in water sources. This work aims at application of dispersive magnetic solid-phase

extraction (DMSPE) for enrichment of PFAS in various surface water samples. Magnetic Fe₃O₄@MIL-101 (Cr) was used as an adsorbent for in MSPE. The concentrations of target analytes in water samples were determined using high performance liquid chromatography-diode array detector and ultra-high performance liquid chromatography-tandem mass spectrometry analysis. The combination of optimized DMSPE with HPLC-DAD and UHPLC-MS/MS provided wide linear range (1-5000 ng/L and 0.05-2000 ng/L, low limits of detection (0.3–0.66 ng/L and 0.011-0.04 ng/L) and limits of quantification (1.0-2.2 ng/L and 0.04-0.12 ng/L). Moreover, acceptable intraday and interday precision based on the relative standard deviation (RSD) lower than 5% were obtained. The developed method showed remarkable practicability for analysis of ultra-trace PFAS in water samples.

8.04.P-Th279 Marine Microbiome Affected by the Discharge of Urban Wastewater Effluent at the Swedish West Coast

Sonja Schaufelberger¹, Alexis Fonseca², Thomas Backhaus³ and Pedro Inostroza⁴, (1)Department of Biological and Environmental Sciences, University of Gothenburg, Göteborg, Sweden, (2)Aarhus Universitet, Denmark, (3)University of Gothenburg, Sweden, (4)Biological and Environmental Sciences, University of Gothenburg, Gothenburg (Sweden), Sweden Marine coastal ecosystems are under increasing anthropogenic pressure. Negative effects due to discharge of nutrients, heavy metals, and organic toxicants from industry, wastewater treatment plants (WWTPs), and/or agriculture not only on macro- and mesofauna, but also microbes are widely recognized. Microbial communities are pivotal players of natural biogeochemical cycles, and thus are vital for the functioning of their ecosystem they inhabit. Hence, any disruption of their ecological functions and/or change in community structure may lead to unwanted disequilibrium. In this study, we used environmental DNA (eDNA) to fill knowledge gaps regarding 1) the biodiversity status of microbial communities, 2) environmental variables driving community structure, and 3) the persistence of sewage-related bacteria in natural environments. Our study site is a fjord system at the Swedish West Coast (Hakefjorden and Havstensfjorden), surrounding the city of Stenungsund, which harbours a variety of industry branches but is chiefly known for being the centre of the Swedish petrochemical industry. Six eDNA samples were collected along the fjords. Additionally, samples from three freshwater inflows were sampled: a WWTP effluent and two streams running through agricultural and urban areas, respectively. Bacterial communities were characterised using 16S rRNA amplicons sequenced on an Illumina platform. State-of-the-art bioinformatic workflows were conducted to obtain amplicon sequence variants (ASVs) and taxonomic assignment (Qiime2 and DADA2). Subsequent downstream analysis was performed using R-packages phyloseq and DESeq2 to assess i.a. α -diversity and differential abundance. Our results showed significantly lower α -diversity in marine microbiomes compared to freshwater ones with about half as many observed ASVs. Interestingly, α -diversity measures were considerably higher at the marine site closest to the WWTP effluent (M5). Conversely, we did not observe α -diversity changes linked to industrial or agricultural freshwater inflows. Communities from marine, freshwater, and WWTP effluent samples were distinct from each other exhibiting a wide range of differentially abundant taxa. Further, environmental variables failed to explain M5 clustering separately from the other marine sites. Overall, we suggest that WWTP may increase the biodiversity in the water column and contribute to shifting the community composition of nearby bacterial communities.

8.04.P-Th280 Modelling the Influence of Climate Change on Contaminant Exposure in Three Key Seabird Species in the European Arctic

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The combined impact of climate change and exposure contaminants on Arctic seabirds remains poorly understood. A mechanistic understanding of the link between global persistent organic pollutant (POP) emissions and exposure in seabirds seems vital to advance our knowledge in light of climate projections. The overall objective of this study is to develop and evaluate a bioaccumulation model and utilize it to predict the impact of climate scenarios on the POP exposure in Arctic seabirds. For this purpose, we further developed the Nested Exposure Model (NEM), a dynamic and spatially resolved environmental fate and bioaccumulation model that simulates the link between global organic contaminant emissions and resulting exposure in key Arctic species. Here, NEM is expanded to include three seabird species with contrasting feeding ecology: the pelagic black-legged kittiwake (*Rissa tridactyla*), the omnivorous scavenging glaucous gull (*Larus hyperboreus*) and the benthic-feeding common eider (*Somateria mollissima*). While NEM already accommodates pelagic species that are key prey for the black-legged kittiwake and glaucous gull, the model was expanded to now also include benthic species that are key prey of the common eider. All seabird species were parameterized based on detailed ecological data, including seasonal lipid dynamics, energy needs and reproductive behavior. To evaluate the model, global historical emission inventories and physicochemical properties of polychlorinated biphenyl (PCB-) 153 are used to predict its concentrations in the three seabird species from 1930 – 2050. Predicted concentrations are then compared to empirical time series of PCB-153 concentrations in the study species sampled in Kongsfjorden, Svalbard. An overview of the NEM model framework, parameterization, and preliminary evaluation will be presented. The model will be used to mechanistically understand and predict contaminant exposure in the study seabirds in different future climate scenarios, e.g. changing primary emissions, changes in the physical environment, or changes in dietary preferences or availability. Ultimately, this study will expand our understanding of the impact of climate change on long-range transported contaminants in Arctic seabirds.

8.04.P-Th281 Morphohistological Data of Normal Variation of the Thyroid Glands From Tadpoles in Amphibian Metamorphosis Assays Using Automated Image Analysis by Machine Learned Algorithms

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Knowledge of the morphological variation of normal thyroid glands in the corresponding developmental stage of tadpoles is a prerequisite for the assessment of possible induced endocrine effects by the toxicological pathologist. Heterogeneity among thyroid glands from control animals, may be associated with larval stage, breeder, facilities, alimentary conditions, possible solvent effects, etc. Histological sections of thyroid glands stained with haematoxylin and eosin from control tadpoles of 12 studies, n = 301 tadpoles stage¹ 57 to 60) from three test facilities (TF1, TF2, TF3) were evaluated by pathologists and the corresponding whole slide images were analysed using an automated procedure in QuPath: Open source software for digital pathology image analysis². In addition, the high dose group from a study with test-item induced hypertrophy was also analysed using the machine learned algorithms to evaluate the use of this tool to support traditional histologic evaluation. The data obtained include total thyroid gland area, ratio between the follicular epithelium vs. total area, height of the follicular epithelium, total colloid area, and ratio between epithelium and colloid area as well as number of epithelial cells. The results provide a basis for understanding normal variation for specific larval stages, and data revealed differences in thyroid gland morphometry among test facilities. Furthermore, there were minor differences between controls in water and controls in solvents. Thus, thyroid findings need to be carefully interpreted and compared to study stage-matched controls for conclusive results. The methodology was also applied to a study with induced thyroid gland hypertrophy and hyperplasia and hyperplasia. Automated image analysis showed marked and significant differences between the control group and high dose for all parameters. It is concluded that image analysis by automated machine learned algorithms enabled objective quantitative data for the thyroid gland and across the complete 2D histological section. Therefore, the image analysis is considered a valuable and accurate tool to support the microscopic evaluation by the pathologist. 1. 1. Nieuwkoop P, Faber J. *Normal Table of Xenopus Laevis (Daudin) : A Systematical and Chronological Survey of the Development from the Fertilized Egg till the End of Metamorphosis*. 2nd ed. Routledge; 1994. 2. 2. Bankhead P, Loughrey MB, Fernández JA, et al. QuPath: Open source software for digital pathology image analysis. *Scientific Reports*. 2017;7:16878.

8.04.P-Th282 Mosaic-Project Extensive Monitoring of Pesticides and PFAS in 274 WATER Bodies in Southern Germany

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The Water Framework Directive (WFD) aims at the achievement of a good ecological and chemical status for all surface waters, which is assessed by monitoring of priority substances and certain other pollutants. In 2018 the MOSAIC-project, a six-year monitoring program, started. The project intends to give a broad overview on the pollution status of Bavarian surface waters, with special focus on smaller rivers. Every year surface water samples of 90 different water bodies are analysed monthly or quarterly for a set of 247 parameters (pesticides, perfluoroalkyl substances (PFAS), heavy metals among other compounds) in total reaching approximately 540 water bodies by the end of project. To complete the picture non-target screening technology is used to identify compounds not focused on yet. The results from 274 water bodies analysed in 2018 – 2020 are presented. Temporal trends and spatial differences in substance patterns are statistically evaluated to identify sources and application mixtures. Concentrations are compared to environmental quality standards (EQS) and sampling sites are correlated to the surrounding land use. The data provided may support the differentiation between background levels and hotspot contaminations. 59 of 71 monthly analysed pesticides were detected. However, the majority of samples did not exceed WFD-quality standards (AA-EQS/MAC-EQS). EQS-threshold exceedances were detected for 10 compounds. Sampling sites were selected based on land use and potential point sources in the catchment area. A broad range from rural to agricultural land uses is covered. The data demonstrate spatial trends of pesticides, which can be related to agricultural land use. 10 PFAS were investigated in four samples a year. PFAS are used in a wide range of applications in different mixtures. Application related patterns could therefore help to identify potential sources. Despite the ubiquitous detection of PFOS, patterns in PFAS mixtures were identified. The statistical analysis indicates the differentiation of two main use categories where PFAS are employed: Fire-fighting foams and industrial applications. While many studies and regulatory actions focus on large rivers, this project provides the unique opportunity to gather a comprehensive overview of compounds present in smaller rivers and streams.

8.04.P-Th283 National Environmental Quality Standards Set in Denmark

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Environmental Quality Standards (EQS) are limit values for substances in the environment set to protect the living organisms in the aquatic environment from possible adverse effects. EQSs are used as tools for assessing the chemical status of waterbodies and to set discharge permits to waterbodies. EQSs set for substances on the Priority Substance List under the European Union's Water Framework Directive (WFD) currently covers 45 substances, cf. directive 2013/39/EU, which have been prioritized for action on a Community level. This directive covers annual average EQS values (AA-EQS) and maximum allowable EQS values (MAC-EQS) for fresh- and marine surface water. And for a few substances the directive covers EQS values for biota, but no EQS values for sediment is covered. The Priority Substance List leaves out substances, which have national relevance for compliance assessments and discharge permits. Thus, in the last couple of years, The Danish EPA has increased the focus on deriving national EQS values for River Basin Specific Pollutants (RBSPs). The focus has primarily been to derive EQS values for sediment in relation to protection of benthic species, in addition to EQS values for biota for the protection of secondary poisoning through the food web, as well as protection of human health from consuming contaminated fisheries products. In the work of setting EQS values for sediment and biota, challenges related to e.g. handling and assessment of available data were encountered. For some RBSPs the amount of reliable data was sufficient to consider and argue for applying a Species Sensitivity Distribution analysis (SSD-analysis) in the derivation of both an EQS value for sediment as well as for biota (secondary poisoning), whereas other RBSPs fulfilled the evidence of e.g. potential to bioaccumulate, which would suggest a derivation of a EQS value for biota, but no reliable and relevant data was available. The list of RBSPs by the Danish EPA covers both organic and inorganic contaminants.

Several EQS dossiers have been finalized and are public available at the agency's webpage. These cover both national relevant substances and substances on the Priority Substance List for which national EQS values for sediment and biota has been set.

8.04.P-Th284 Natural Polyphenolic Extracts As Antioxidants for Biopolymers

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In the plastic field, a major challenge is to substitute synthetic additives with greener chemicals with the intent to develop environmentally friendly materials. The development of natural additives, including stabilizing agents, is increasingly investigated for polymer materials and particularly for biopolymers. All through their lifecycle, polymers are subject to degradation, such as thermo- and photo-degradation, leading to the deterioration of their properties. Stabilizers are used to prevent and/or slow down the degradation kinetics and control the durability of polymers. Recently, bio-sourced antioxidants, such as polyphenols, have demonstrated promising results for the protection of polymers against degradation and aging. This study is a part of the Horizon 2020 project SEALIVE, whose purpose is to reduce plastic waste and environmental pollution of land and seas by developing innovative and sustainable bioplastic strategies contributing to the circular economy. The objective is to elaborate various demonstrators from new bioplastics solutions developed within SEALIVE. This work presents the thermal and photo-stabilization of poly lactic acid (PLA) biopolymer with polyphenol extracts from biomass valorization. As a first step, an initial evaluation of the antioxidant activity of the extracted polyphenols from different vegetal matrices (agri-food by-products, industrial residues, wild plants) has been performed. As a second step, thermal and photo stabilization studies were conducted on PLA formulated with the polyphenols exhibiting high antioxidant activities. The stabilizing efficiency of the extracts was mainly evaluated using rheological analysis to monitor the molecular weight evolution during processing and the exposition to thermal and photo-aging in accelerated conditions. The results demonstrated that different polyphenol extracts successfully delay PLA degradation and constitute very promising natural stabilizers. **Acknowledgments** *The work in this paper is supported by the SEALIVE Project which is funded by the European Union's Horizon 2020 Program, under grant agreement number 862910.*

8.04.P-Th285 Optimization of White-Rot Fungi Mycelial Culture Components for Bioremediation of Pharmaceutical-Derived Pollutants

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The issue of the presence of pharmaceuticals in the aquatic environment emerged about 15 years ago, but recently more and more data have been collected on their adverse effects on biota. The occurrence of PhACs in the aquatic environment can be caused by improper disposal processes applied by producers or leachate from landfills, farms, nevertheless, wastewater treatment plant (WWTP) effluents contribute the most. Although WWTPs are in general able to remove organic pollutants, the effectiveness of this process varies. Currently, in the treatment of sewage with the activated sludge method, numerous microorganisms are used, mostly bacteria. Nevertheless, these microorganisms are not resistant to many drug contaminants. Moreover, PhACs pollutants accumulating in water environment may also pose a risk to human health. White-rot fungi are promising candidates to solve this problem, because they degrade a wide spectrum of environmental pollutants, including pharmaceuticals, which are not efficiently removed from wastewater by using conventional methods. Numerous studies showed that mycoremediation offers a more effective approach to wastewater treatment. Now, mainly high costs of media ingredients limit the application of mycoremediation on a large scale. We aimed to screen 18 media ingredients, including seven agri-food by-products to select low-cost medium optimal for biomass production for three selected white-rot fungi. The submerged cultures of *Armillaria mellea*, *Phanerochaete chrysosporium* and *Pleurotus ostreatus* were conducted in different media ingredients such as: ammonium salts, corn waste products, molasses, saccharides, sawdust, soy peptone, urea, whey, yeast extract. The reference medium contained glucose, casein peptone, yeast extract and potassium dihydrogen phosphate. Three media variants provided efficient growth, i.e. corn steep liquor, dried distillers grains with solubles and whey. The results could be used in further research towards designing a plan to implement agri-food products for white-rot fungi cultivation in municipal sewage.

8.04.P-Th286 Organophosphate Esters in the Water Column and Surface Microlayer of the Atlantic Ocean

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Organophosphate esters (OPEs) are synthetic organic chemicals widely used as plasticizers and flame retardants in the industry, in household consumer products and in personal care products. Their range of physicochemical properties is wide, influencing their transport and biogeochemistry in the marine environment. Our goal was to assess the spatial and vertical distribution of OPEs by sampling and analyzing samples taken during a latitudinal transect through the Atlantic Ocean (from latitude 40 °N to 55 °S). Surface seawater was analyzed from 24 sampling stations, while the surface microlayer (SML) and vertical profiles (5-6 different depths from surface to 2000 m depth) were assessed at 9 and 7 stations, respectively. A total of 24 individual OPEs were targeted, among which 13 compounds were found ubiquitous in Atlantic waters (TEP, TiBP, TNBP, TCEP, 1, 2, 3-TCPP, TDCIPP, TPhP, TBEP, EHDP, TEHP, TmCP). Briefly, the surface total concentration for the dominant OPEs ranged between 1.9 and 56.9 ng L⁻¹ (individually TEP: loq to 4.1 ng L⁻¹, TiBP: loq to 5.6 ng L⁻¹, TNBP: loq to 1.5 ng L⁻¹, TCEP: loq to 10.9 ng L⁻¹, TCPP1: 1.2 to 32.8 ng L⁻¹, TCPP2: 0.2 to 19.1 ng L⁻¹, TCPP3: loq to 0.5 ng L⁻¹, TDCIPP: loq to 0.7 ng L⁻¹, TPhP: loq to 1.3 ng L⁻¹, TBEP: loq to 3.1 ng L⁻¹, EHDP: loq to 7 ng L⁻¹, TEHP: loq to 0.3 ng L⁻¹, and TmCP: loq to 0.5 ng L⁻¹). Maximum concentrations were found in tropical waters of the SE Atlantic off-shore Brazil. At the SML, the top 0.1 mm layer at the air-water interface as sampled with a

glass plate, we found higher OPE concentrations when compared to underlying waters by SML enrichment factors ranging between 1.2 to 24. Therefore, OPEs show a tendency to accumulate at the surface of the ocean, as it has been demonstrated for other organic pollutants, with implications for their potential long-range transport through sea-spray aerosol formation. Regarding the vertical profiles, we have found different trends depending on the locations, but generally with maximum concentrations at surface waters, consistent with atmospheric deposition as one of the main inputs. However, a second maximum was often found at deep waters, which suggests that vertical transport by settling particles may lead to the accumulation of OPEs in the deep ocean. The results will be shown in connection with other physical, chemical and biological variables explaining their transport and water column biogeochemistry.

8.04.P-Th287 Persistent, Mobile, and Toxic Substances - Evaluation of Risk to Drinking WATER in England and Wales
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Substances that are persistent and mobile have a greater potential to pass natural barriers like riverbanks and migrate to groundwater. Such substances also have a greater propensity to breakthrough in standard drinking water treatment because of their physico-chemical properties. Substances with these intrinsic properties, especially when considered in combination with toxicity may pose a risk to human health and the environment through contamination of drinking water sources. In recent years there has been an increasing body of work to explore data criteria for the definition of a hazard class relating to persistent, mobile, and toxic substances with the aim of protecting natural resources that could be used for drinking water supply. In Europe, substances that are persistent (P), mobile (M) and toxic (T) or very persistent (vP) and very mobile (vM) have been proposed as an additional hazard class under REACH and the Classification, Labelling and Packaging regulations. In 2020 the European Commission published its 'Chemicals Strategy for Sustainability Towards a Toxic-free Environment' which commits to the introduction of PMT and vPvM as categories of substances of very high concern. We describe an ongoing project to assess the risk to human health from PMT/vPvM substances through exposure via drinking water. The project focuses on drinking water sources in England and Wales and examines the risks posed by approximately 30 potential PMT substances. Toxicological data has been collated to understand the hazards associated with each substance. Data and intelligence to establish the nature of use and quantities used in England and Wales and evidence on occurrence in environmental waters in England and Wales has also been gathered. Modelled surface water concentrations will be used to generate exposure maps for England and Wales. Exposure predictions for humans via drinking water will be estimated from predicted raw water concentrations after accounting for removal of the compounds of interest by standard and advanced treatment processes, based on their physical chemical properties. A spatially based assessment of risk will be made to identify those substances likely to pose the highest risk to human health through drinking water. Potential risk factors that could be used to inform the development of future targeted risk-based monitoring programmes and identify any high-risk catchments will be identified.

8.04.P-Th288 Prospective Comparison of the Primary and Secondary Production of Nd-Fe-B Magnets
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Due to the increasing adoption of wind turbines and electric motors for battery-electric mobility, the energy transition requires an increasingly secure supply of permanent rare earth magnets. While the current supply chain of rare earths heavily relies on mining them in China, Nd recycling outside of China is expected to play a prominent role in the future. One of the alternative recycling processes currently under development in the framework of the Horizon 2020 project "SUSMAGPRO" involves the use of hydrogen to retrieve Nd-Fe-B from end-of-life magnets. We performed an LCA resulting in a prospective comparison of the environmental profile of the hydrogen-based recycling alternative and the primary production of permanent magnets, in collaboration with the developers of the recycling technology. This recycling technology currently operates at pilot scale; however, the environmental profile we derived corresponds to an extrapolated scenario of a higher technological maturity. We included, in the product system models, future trends in the energy systems up to 2050. We found that the hydrogen-based recycling of magnets showed a better environmental performance than the primary production at industrial scale in all the impact categories evaluated. One of the key drivers of this difference is the reduced energy demand from reprocessing the alloy instead of chemically reducing the metallic oxide extracted from the ore. The future trends in the energy systems we considered indicate a lower environmental impact of electricity. As a consequence, the environmental burdens of the primary production would decline but the environmental burdens of the secondary production would decline even faster. After discussions with the developers of the recycling process, we also identified environmental hotspots and advised them on how to optimize the technology and the sustainability of the alternative recycling process.

8.04.P-Th289 Read-Across for the Chemical Safety Assessment: Possible Approaches, Challenges and Perspectives
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The past few decades have seen unprecedented growth in the synthesis of organic and inorganic chemicals. These are then marketed and can be found in the environment. Although the majority of industrial chemicals are designed to be biologically inactive, there are numerous examples of chemicals causing adverse environmental effects years after they've been produced. On

the other hand, with an ever-increasing number of synthetic chemicals being manufactured, it is unrealistic to expect that they will all be subjected to comprehensive and effective risk assessment. The EU REACH legislation, the OECD and US EPA official guidance documents, and 3Rs principle (Replacement, Reduction, Refinement of animal testing) all advocate the necessity of developing comprehensive computational methods (e.g.: read-across methods) that would enable the predictive modeling of both chemical-specific functionalities and their hazard. A shift from conventional animal testing to computer-aided methods is, therefore, an important step towards advancing the environmental risk assessments of chemicals. These tools would also be crucial in establishing *safe-by-design* principles at the early stages of new chemical development. Based on the experience of SONATA-12 project, funded by the Polish National Science Centre (UMO-2016/23/D/NZ7/03973) we will present the current state-of-the-art of the *in silico* methods, with the focus on read-across models. Then, we will demonstrate the examples of read-across models for predicting the acute toxicity of structurally diverse organic chemicals to fish and aquatic invertebrates. Finally, we will discuss a further perspective for the development of computational toxicology.

8.04.P-Th290 RobustNature Excellence Cluster Initiative - Robustness and Resilience of Nature-Society Systems in the Evolving Anthropocene

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Our planet is facing existential challenges such as the decline of biodiversity, climate change and chemical pollution. Though comprehensive research is being conducted to study the individual aspects, especially the interactions and interdependencies of these three areas on a global scale are identified as major challenges for international research in the coming years. The higher-level system functionalities are highly dynamic processes, and their constant changes as well as their robustness and resilience are neither well understood nor quantified. We would like to present the RobustNature Excellence Cluster Initiative, which aims to improve the understanding of robustness and resilience of Nature-Society Systems in the contexts of chemical pollution, biodiversity loss and climate change by means of knowledge-based transformation research. Multiscale approaches from molecules to ecosystems in the three research areas Water, Organismic Interactions and Systemic Risks are used to identify markers for multiple stressors and their specific effects. Furthermore, economic and societal impacts are investigated and strategies to manage and avoid the risks stemming from imbalanced nature-society systems are developed. We believe that such a holistic approach needs to combine interdisciplinary expertise from biology, geosciences, environmental sciences, social sciences, law, economics, and medicine to cover ecological, economical, and social perspectives. Thus, the RobustNature consortium, a large collection of various departments from Goethe University Frankfurt am Main (Germany) as core institution as well as international partners from academia and industry invites researchers for collaboration, expert dialogues, and scientific exchange. RobustNature is an initiative to establish a cluster of excellence with a long-term perspective, which we believe is crucial to tackle the challenges of our modern society. As an innovative method to successfully implement the research questions and to strengthen interdisciplinary collaborations, several SynergyFund projects have been initiated in RobustNature. In a first phase, 11 joint projects are successfully established. Projects are characterized by the funding of scientists in early career stages, by the participation of many working groups and (inter)national external partners.

8.04.P-Th291 Role of Dissociation Kinetics in Determining Platinum and Palladium Toxicity to a GREEN Alga and Their Accumulation

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Ever since the introduction of platinum (Pt) and palladium (Pd) in automotive catalysts, the motor vehicle exhaust has become the primary source of emission of those metals in the environment. Although initially believed to be inert, several studies have recently demonstrated their mobility. Once mobilized, they are likely to reach aquatic ecosystems, where both Pt and Pd complexes will undergo speciation changes. The kinetic process can be predicted by the water exchange rate which, for many transition metals, is in the order of 10^{-7} to 10^{-10} s⁻¹. For Pt and Pd, the rate constants are orders of magnitude higher (around 10^3 s⁻¹), which means that speciation changes may take place over a relatively long-time scale. In this project, we examined the accumulation and toxicity of Pd and Pt using the green alga *Pseudokirchneriella subcapitata* as a function of their initial forms (i.e. as amine or chloride complexes) and potential re-speciation during the exposure experiments. The microalgae were exposed for 96 h in a modified USEPA medium containing either the chloride or amine complex of Pt(II) or Pd(II). We hypothesized that, if these metals quickly reach thermodynamic equilibrium, they should mainly re-speciate into hydroxo-complexes and exert similar toxicity irrespective of the initial form. Similar growth inhibition thresholds (EC50s) and levels of Pd accumulation were observed for both complexes. However, tests with chloro-Pt showed higher toxicity than with amine-Pt. Surprisingly, similar accumulation levels of Pt were observed, indicating a disconnect between accumulation and effects. These results thus suggest that the changes in Pd speciation occur within a time frame short enough to provide similar toxicity to the green alga while Pt speciation occurs slowly and toxicity differs based on the initial speciation. Changes in metal speciation over time for all complexes tested was further investigated by UV-visible absorbance to get insight on the time scale of these changes. Changes in spectrophotometric spectra were observed within hours for chloro-complexes of Pt but not for amine-complexes, suggesting a higher stability of amine-Pt complexes. Our work shows that the initial form of Pt(II) used in toxicity tests needs to be considered

and that thermodynamic equilibrium cannot be assumed to be reached over the exposure time. On the other hand, the toxicity of Pd(II) is likely governed by the resulting chemical speciation at equilibrium.

8.04.P-Th292 Species Sensitivity Distribution in Ground Beetle Communities Inhabiting Oilseed Rape Fields Towards Three Insecticides

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The ever increasing pesticide use in agriculture is considered one of the most important causes of insect decline worldwide. Among non-target invertebrates particularly endangered by pesticide sprays are ground beetles – an important group of Ecosystem Service Providers (ESPs) in agro-ecosystems, acting as natural enemies of crop pests. To understand how pesticides may affect ecosystem services provided by carabids it is crucial to assess effects of exposure not only at the individual level, but also for whole communities. The objective of our study was, thus, to assess the distribution of sensitivity in ground beetle communities inhabiting oilseed rape fields towards three insecticides: lambda-cyhalothrin, acetamiprid and chlorpyrifos. Using a vial test, in which an active ingredient dissolved in acetone is distributed evenly inside a glass vial, we first assessed acute toxicity of each insecticide to 7-14 species. The median lethal doses (LD₅₀) for each active ingredient, estimated using probit analysis, were used to establish Species Sensitivity Distribution (SSD) curves by fitting cumulative distribution functions to the species-specific 48h-LD₅₀ data. The SSD curves were further used to estimate the Potentially Affected Fraction (PAF) of species at doses of the active ingredients recommended for field use and to derive Hazardous Dose for 5% species (HD₅). PAFs of species at one recommended field dose were ca. 75% for acetamiprid and almost 100 % for lambda-cyhalothrin and chlorpyrifos. The estimated HD₅ values for acetamiprid, lambda-cyhalothrin and chlorpyrifos constituted 0.047 (95% confidence interval (CI): 0.016 ? 0.155), 0.006 (95% CI: 0.002 ? 0.020) and 0.002 (95% CI: 0.001 ? 0.004) of their recommended field doses, respectively. Although the threat to ground beetle communities in oilseed rape crops was the lowest in case of acetamiprid and the highest for chlorpyrifos, the dose of acetamiprid approx. 20 times lower than the recommended dose is already dangerous for beetle communities. Our results also revealed a high level of variability of the sensitivity in the set of species tested, with *Bembidion lampros* being one of the most sensitive species to all insecticides. This study was supported by the European Union's Horizon 2020 research and innovation programme, under grant agreement no. 773554 (EcoStack)

8.04.P-Th293 Structural and Functional Changes in the Wheat Rhizosphere Bacterial Community Upon Copper Nanomaterials Application to Soil

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The application of (nano)pesticides in agriculture might lead to the exposure of rhizosphere to copper-based nanomaterials, possibly affecting several bacterial-mediated processes. Thus, we investigated the effects of copper-based compounds [Kocide®3000, nCu(OH)₂, nCuO and Cu(OH)₂-i (as an ionic control), at 50 mg kg⁻¹ soil] on the function and structure of rhizosphere bacterial communities from *Triticum aestivum* L., using indoor mesocosms which included other soil organisms: *Eisenia andrei* (ten individuals), *Porcellionides pruinosus* (ten individuals), and *Tenebrio molitor* (ten individuals), during 28 days of exposure. The soil located outside the rhizosphere, i.e., bulk soil, was analyzed for comparison purposes. Overall, the structure and function of the rhizosphere bacterial community were affected by copper exposure, mainly at day 14. At this exposure time, enzymatic activity related to the nitrogen, phosphorous, and sulfur cycles significantly decreased in copper-treated rhizosphere soil when compared with control rhizosphere soil. At the structural level, a reduction of alpha-diversity was observed in nCu(OH)₂-treated rhizosphere soils, at day 28. Additionally, the structure and function of bulk soil bacterial community were affected by copper, but only at day 28, suggesting a stimulation of copper dissolution by the root exudates. In general, the ionic Cu(OH)₂-i exerted a similar impact compared to the nano-formulations. Concerning nanomaterials, nCu(OH)₂ (structure) and nCuO (enzymatic activity) presented a stronger effect on the rhizosphere bacterial community. Thus, this work highlights the relevance to study the rhizosphere soil to assess the impact of copper-based nanomaterials in terrestrial compartment. Further studies should clarify the role of exudates as a driver of the rhizosphere microbiome and as a determinant of copper dissolution in soil.

8.04.P-Th294 Sub-Lethal Exposure to Teflubenzuron Leads to Disrupted Feeding Rates in *Daphnia magna*

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Benzoylureas are chitin synthesis inhibitors (CSIs) used as larvicides in agriculture, aquaculture and pest control. The wide use of CSIs leads to increased exposures to non-target organisms in surrounding aquatic ecosystems. However, the effects of CSIs are poorly studied on non-target organisms like filter feeding cladocerans. Physiological endpoints, such as feeding activity are expected to be sensitive indicators of CSI effects, due to possible changes in the functionality of the chitin containing peritrophic membrane (PM) in the midgut. As the standard acute toxicity tests require the absence of food during exposure, there is a need for data describing molecular level responses in environmentally representative scenarios. The aim of the study is to quantify the sub-lethal effects to the integrity of PM due to reduced chitin synthesis and the subsequent effect on feeding rate and growth of *D. magna*. To test the hypothesis that at sub-lethal levels of CSI exposure, the toxic effects are primarily expressed as disruption of

PM integrity and consequential reduction in feeding, *Daphnia magna* juveniles (4-5 days old) were exposed to sub-lethal concentrations of teflubenzuron (0,005-2 µg/L) for 48 to 72 h to evaluate its effect on the feeding behavior, expression of chitin synthase 2 (*CHS2*) in midgut and the chitin content of PM. Feeding behavior was assessed as clearance rate (CR) of algae *Chlamydomonas reinhardtii* every 12 h measuring the cell count in flow cytometer, *CHS2* expression was measured by qPCR using non-specific *CHS* and targeted *CHS2* primers, chitin content in the PM was quantified with colorimetric analysis after wet digestion in KOH and chitinase. Only the highest teflubenzuron concentration showed consistently lower CRs (30-60% of control) over the entire duration of exposure. The dynamic changes of CRs in lower concentration exposures were attributed to the toxicity mitigating effect of food and bioavailability of teflubenzuron as the CR recovery showed a dose-dependent effect toward the end of exposure. The changes in PM chitin content and the expression of *CHS2* in gut epithelia according to the suspected mode of action of benzoylureas are presented. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 859891 and was supported by NIVA's Computational Toxicology Program (NCTP, www.niva.no/nctp).

8.04.P-Th295 The Brigid Project: Bridging the Gap Between Microplastics and Human Health

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While plastics application provides benefits that contribute to a safe and sustainable society, widespread plastic pollution has been recorded in many ecosystems on Earth. This has been associated with measurable microplastic (MP) presence across the globe, leading to unintentional biota and human exposure. Currently, there is no clear consensus from scientists and regulatory bodies over MP adverse health effects. However, large knowledge gaps still exist within this research topic, which need to be urgently addressed with high-quality scientific data. Moreover, previous studies often employ experimental conditions relying on unrealistic exposure scenarios, including testing higher concentrations, or narrow size distributions, and using virgin MPs. In this context, Plastics Europe, the pan-European plastics manufacturer trade association, is launching a large-scale five-year scientific research project: **Brigid**. Brigid aims to assess the potential risks to human health from MP exposure via ingestion (one of the major hypothesized routes of human exposure, together with inhalation). To deliver relevant results, Brigid focuses on secondary MPs of polyethylene (both linear low density & low/high density), polypropylene, polystyrene, polyvinyl chloride, polycarbonate, and polyamide-6, which are representative of higher production volume polymers currently produced.

The initial planned project work and communication activities include: i) creation and characterisation of secondary MPs through milling in relevant size ranges. Some of the MPs will also be labelled to help with exposure studies; ii) quantification of potential MP human internal exposure through ingestion, with *in vitro* and *in vivo* data to support an advanced exposure assessments with *in silico* PBPK model; iii) in-depth MP hazard characterisation using an integrated approach, starting with *in vitro* human cell models, as well as innovative *ex vivo* testing on organoids, and *in vivo* testing with animal models; iv) development of a hybrid Risk Assessment framework by combining *in vivo*-based and *in vitro* assessment approaches; v) active stakeholder dialogues with different interest groups to facilitate open and transparent communication of results to support informed policymaking. Further, Brigid works closely with other industry-funded projects to develop a coordinated global MP research program addressing identified data gaps: the ICCA Microplastics Advanced Research and Innovation Initiative (MARII).

8.04.P-Th296 The Early Environmental Safety Approach

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Chemical plant protection products are crucial to meet growing agricultural demands in the 21st century. However, novel plant protection products (PPP) need to meet rigorous societal and regulatory standards. The optimal balance between a high efficacy to lower application rates and a minimal environmental footprint, (e.g. off target toxicity in pollinators & persistence in the environment), challenges the development of novel molecules. To cope with this challenge, rapid and reliable screening of environmental profiles of novel PPP candidates, combined with tailored *in silico* solutions, is already needed in early developmental phases to identify and prioritize candidates with a high success rate. Within early Environmental Safety (eEnSa), we develop integrated automated high-throughput environmental screening assays for a wide range of safety dimensions ranging from classic hazard assessment to advanced soil persistent screening and bioaccumulation modeling. These capabilities will enable to rapidly identify and consequently focus on developing the next generation of only the most environmentally compatible PPPs with a high probability of societal and regulatory acceptance. This integrated eEnSa approach will ultimately enable to select compounds with improved favorable environmental safety profiles and re-shape the selection and optimization of promising novel chemical starting points to develop the next generation of more sustainable plant protection solutions. This poster presentation will introduce the concept and application of the eEnSa strategy and aims to serve as a starting point to facilitate a discussion with the wider SETAC community about the challenges and opportunities of shaping the next generation of plant protection products.

8.04.P-Th297 The Potential of Emerging Bio-Based Products to Reduce Environmental Impacts [Under Review]

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Moving from a fossil-based to a bio-based economy is considered an important strategy to mitigate climate change. However, to

ensure that bio-based products will contribute to a more sustainable economy, environmental assessments at early developmental stages are needed. Prospective Life Cycle Assessment may support the evaluation of emerging bio-based products and unveil possible trade-offs. We reviewed 112 papers and retrieved data related to the environmental impacts of 86 emerging bio-based products. To compare the results, a data curation was needed, namely harmonizing system boundaries, functional unit, end-of-life treatment and biogenic carbon accounting across all prospective life cycle assessments. We find that the greenhouse gas (GHG) footprints of emerging bio-based products are on average 47% lower (95% confidence interval (CI): 38, 54%) compared to their fossil alternatives. Grouped in product categories, biorefinery products show the highest predicted GHG reduction with 73% (95% CI: 50, 85%). Non-renewable energy demand is also reduced with 30% (95% CI: -58, 8%), however, emerging bio-based products have on average 32% (95% CI: -29, 143%) and 359% (95% CI: 109, 906%) higher acidification and eutrophication impacts compared to their fossil-based alternatives. Most emerging bio-based products can thus make an important contribution to reaching GHG emission reduction targets, but tradeoffs with other environmental impacts should not be overlooked.

8.04.P-Th298 Towards Environmentally Relevant Biodegradation Kinetics for Chemicals Emitted From Offshore Oil Platforms

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Persistency of chemicals emitted to the environment is commonly evaluated using data from standardized laboratory biodegradation tests. The conditions of these tests can vary greatly from the real environmental conditions. Research is needed to bridge the knowledge gap between the biodegradation kinetics predicted from standardized laboratory tests and the actual environmental biodegradation kinetics. Produced water from offshore oil and gas production is continuously discharged to the sea in high volumes. Produced water from a platform in the North Sea is used as a case study in this research. The aims are to (1) develop an approach to improve environmental relevance of biodegradation kinetic testing and (2) investigate the effect of dilution of produced water in seawater on biodegradation kinetics. Produced water (emitted chemicals) is combined with seawater (native degrader microorganisms) in a novel biodegradation test. A series of parallel experiments at different dilution levels will run for 60 days, testing at environmentally relevant concentrations and at the sampling temperature of the seawater. During the experiments, biotic (seawater) and abiotic (ultrapure water) test systems will be analyzed using SPME coupled to GCMS, to obtain relative substrate depletion. This data is then used for biodegradation kinetics estimations. The experiments will run in April and May 2022 and preliminary results will be presented. Methods for environmentally relevant biodegradation kinetics testing of emitted chemicals can improve the site-specific risk assessment and give information on chemicals of concern when aiming at a reduced pollution society.

8.04.P-Th299 Toxic Reference Substances for Soil Organism Studies Performed According to OECD and ISO Guidelines: Are There More Safe Alternatives?

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To ensure environmental safety in the process of registration of agrochemicals, or to investigate the toxicity of contaminated soils, surrogate soil organisms are tested according to OECD (Organization for Economic Co-operation and Development) or ISO (International Organization of Standardization) guidelines. As an integral part of the validity criteria in each OECD/ISO guideline, testing of a toxic reference substance (positive control) should provide assurance that test conditions and sensitivity of test organism strains used in the specific laboratory or of species observed in the field are adequate. The effects observed with the reference substances should fall into a given range to indicate that the lab test strain or the field species show an appropriate sensitivity. We reviewed the safety, availability, and effect characteristics of the currently suggested toxic reference substances used for soil organism testing. The toxic reference substances suggested in the OECD/ISO guidelines for the surrogate soil organisms *Eisenia fetida/andrei*, *Folsomia candida* and *Hypoaspis aculeifer* are older pesticides (carbendazim, dimethoate, phenmedipham), biocides (chloracetamide) or basic chemicals (boric acid). We compared these substances regarding their worker safety, availability, and effect characteristics in laboratory tests. Based on European Chemicals Agency (ECHA) data, apart from phenmedipham, all suggested substances (especially boric acid) are labelled as highly toxic being either reprotoxic, carcinogenic, mutagenic, and/or volatile. They should, if possible, be substituted in new guideline versions. It becomes increasingly difficult to order the older pesticides since due to their toxicity and environmental profile they are no longer registered in many countries. We present our own studies with *H. aculeifer* comparing the effects (toxicity and dose response curve) of dimethoate, boric acid, and two potential reference substances chlorpyrifos-methyl and copper chloride (CuCl_2). CuCl_2 appears as a suitable alternative for *H. aculeifer*. Laboratory tests from literature performed with CuCl_2 showed also for *F. candida* and *E. fetida/andrei* good dose-responses in reproduction tests. We conclude that CuCl_2 would be a promising candidate for a substitute of the current reference substances since it exhibits low toxicity to humans, is cheap, is readily commercially available (as basic chemical) and its dose response curves appear suitable.

8.04.P-Th300 Until We Know Better, Suggestion of Mixture Allocation Factors (MAFs) Based on Human Blood Exposure

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In the Anthropocene, all living organisms are exposed to multiple anthropogenic organic contaminants (OCs) at once. The combined chemical mixture effect is currently unknown. Different public health issues, such as autoimmune diseases and reproductive disorders can only be genetically explained by a third of the incidences. Other reasons potentially influencing the incidences are viral infections, stress and chemical exposure. When performing a risk assessment (RA) of an OC entering the market today, there is no consideration for unintentional mixtures, even though REACH is underpinned by the precautionary

principle (PP). Furthermore, when a mixture of OCs is assessed the lack of comparable toxicity data is evident. This study offers a guidance on how to address unintentional chemical mixture exposure when sufficient data is not available for a RA. In a recent project, an in-house database (Human Blood Database, HBDB) was created, and used for an exposure assessment for the Swedish general population. Human biomonitoring guidance values (HBM-GVs) were used for the RA. Only a first-tier mixture RA could be made since the reference values available had different critical endpoints. Also, only 9 HBM-GVs could be found for the OCs found in blood of the Swedish population. According to HBDB, 440 OCs have been reported in human blood world-wide. In this guidance, we include all potential OCs in the exposure assessment, which could be considered a worst-case scenario as some OCs might not constitute a risk. Using different time ranges of sample analysis, and different choice of model drivers, six scenarios were constructed. They were created to estimate the range of mixture allocation factors (MAFs) that could be used when assessing the risk of individual chemicals. The scenarios are presented with all their limitations as transparently as possible to highlight the uncertainties. Only OCs in blood were considered, no urine contaminants and no inorganic contaminants. The MAFs derived for each scenario ranged between 15-200. Since little is known regarding the risk associated to complex mixtures, the PP should be applied. Adding a MAF of 200 during risk assessment of new OCs entering the market would theoretically protect the general population from public health diseases. The MAF should be subject to review when new scientific information becomes available, encouraging testing and innovation.

8.04.P-Th301 Wastewater-Based Epidemiology Monitoring of Drug Use and Chemical Exposure During COVID-19 in Athens, Greece, Utilising Wide-Scope Lc-Hrms & LC-MS/MS Target Screening Analysis

Eleni Aleiferi, Aikaterini Galani, Aglaia Gkogkou, Aikaterini Kontou, Maria-Christina Nika, Nikiforos Alygizakis and Nikolaos Thomaidis, National and Kapodistrian University of Athens, Greece

Wastewater-based epidemiology monitoring of drug use and chemical exposure during COVID-19 in Athens, Greece, utilising wide-scope LC-HRMS & LC-MS/MS target screening analysis Aleiferi E., Galani A., Gkogkou A., Kontou A., Nika M.C., Alygizakis N., and Thomaidis N.S.*Laboratory of Analytical Chemistry, Department of Chemistry, School of Natural Sciences, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Greece* Corresponding Author After the outbreak of **COVID-19** pandemic, countries around the world proceeded with the implementation of restrictive measures as key strategies for coping with the pandemic transmission. Strict containment measures such as extensive quarantine during the COVID-19 pandemic, in combination with the psychological pressure that the pandemic itself might have inflicted in the general population, may have affected **mental health, human lifestyle and behaviour**. As a result, changes in patterns of drug and chemicals use might have occurred. These changes can be monitored by the utilisation of the emerging field of **wastewater-based epidemiology** (WBE). The continuously growing field of WBE aims at observing potential consumption and use trends of chemicals in community levels via the determination of biomarkers such as pharmaceuticals, illicit drugs, antipsychotic drugs, surfactants, biocides, personal care products and other chemicals through the analysis of influent wastewater samples. The focal scope of this study was the reflection of potential pattern changes in chemical exposure and drug use in Attica, Greece during the COVID-19 pandemic after the second wave. For this purpose influent wastewater samples were collected on a daily basis and analysed from January 2021 onwards. A total of four or five pooled samples per month were prepared for each week of the pandemic. A generic sample preparation and a wide-scope LC-ESI-QTOF-MS analytical method, in both positive and negative polarities was employed, for the determination of > 2000 chemicals from various category classes. In addition, a LC-MS/MS method was applied for a total of 180 substances, due to its great sensitivity for specific analytes of interest. The detected analyte concentrations were back-calculated using daily flow rates for the investigation of potential load and consumption trends. The drugs and chemicals' use reflected pattern changes during the studied months. Some of the results may be linked with the pandemic and its effects on the population of Attica.

8.04.P-Th302 What Makes a Sustainable Chemical Product? Case Study of Supporting Domino's Ink and Printing Solutions With LCA

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1. Challenge Domino is a global printing solutions provider, supporting clients with printers and inks across industries such as food & drink, life sciences and personal care. Domino's clients and its end consumers are increasingly sustainability focused and are asking Domino to evidence the sustainability credentials of its products. As a result, Domino needs a way to robustly quantify the sustainability impact of its products, from its printers to the different chemical compositions of its ink choices – this is where life cycle assessment (LCA) can help. 2. Solution Domino appointed Ricardo Energy & Environment to develop a LCA tool that could model its printers using different ink types under a specified user-defined scenario. The tool enables Domino to assess the environmental impacts of its products for a specific customer from cradle to grave. It can analyse one specific printer; 10 ink formulations; various use scenarios and 21 environmental indicators, from global warming potential to water use to ozone formation and many more. 3. Outcome The LCA tool is a differentiator in the market and sets Domino apart from other printing companies as a sustainable company who take environmental impacts seriously. The LCA enables Domino to talk to its clients about the sustainability impacts associated with a specific customer's specific operation, accounting for geography, print runs, ink composition and more. The tool has demonstrated the importance of thinking beyond carbon, as solutions that yield the lowest global warming potential were found to result in the highest impact in other environmental indicators. Domino now has a tool to assess the wider environmental issues, helping to target improvements in areas that will have the greatest sustainability impact and avoid improving one impact criteria to the detriment of another.

8.04.P-Th303 Where Are the Microplastics? The Abundance and Distribution of Microplastics in the Waters and Organisms of the River Thames, UK

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Microplastic contamination of the global environment is a growing issue of great concern. Freshwater systems, specifically rivers, are key in the transportation of terrestrially derived microplastics into the marine environment and it is crucial to understand the abundance, distribution and biological effects of microplastics within river systems. The River Thames is the second largest river in the UK and has multiple anthropogenic stressors and pathways of microplastic contamination along its trajectory. This study aims to determine the distribution and abundance of microplastics in the surface waters and benthic organisms of the River Thames. It explores how location and proximity to points of contamination can affect dispersal of microplastics and how feeding mode might influence the ingestion of microplastics in benthic dwelling organisms. Water samples were collected from the river in May 2019 along with benthic invertebrates from three sites with points of potential contamination. Initial findings reveal a high abundance of microplastics which increases along the trajectory of the river (1.05 mp/m³ at the furthest upstream sampling site increasing to 5.72 mp/m³ at the lowest downstream sampling site). Points of suspected contamination to the river influenced the distribution of microplastics along the trajectory. In all sites sampled, fragments and fibres were the most dominant particle shape. In benthic invertebrates, filter feeders ingested the highest abundance of fibres whilst grazers ingested the highest abundance of fragments. The abundance of particles ingested by invertebrates varied across study sites showing differing levels of contamination. The presence of microplastics in a range of benthic taxa aligned with differences in dominant particle shapes in species with distinct feeding modes indicates widespread contamination with potential ecological impact of microplastics in freshwater species of the River Thames.

8.04.P-Th304 Differential Impact of Brominated and Organophosphate Flame Retardant POPs on Gene Expression in Monocyte-Macrophage Differentiation Models

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Several groups of flame retardant chemicals have recently been classified as persistent organic pollutants (POPs), while others are under close ecotoxicological scrutiny. Immunotoxicity and immunomodulation are two of their modes of deleterious action that are insufficiently comprehensively studied. Signalling pathways and gene expression patterns during immune cell differentiation are the most vulnerable site of deleterious xenobiotic action. We have verified the impact of flame retardants from different chemical groups (brominated ethers, brominated phenols, organophosphates and chlorinated organophosphates) on a cellular model of the human monocyte-macrophage differentiation axis which is crucial for effective innate immunity. We applied three cell lines which differ in advancement of the differentiation process (HL-60, THP-1 and MonoMac6) as well as their pharmacologically stimulated macrophage-like derivatives. We measured the effect of flame retardant exposure at environmentally relevant concentrations on levels of gene expression of crucial differentiation markers, immune activity effectors and important signalling pathway elements. Our results point to two main conclusions: a higher vulnerability of less differentiated cells (myeloid precursors and monocytes) to immunomodulatory action of POPs, and a pronounced difference in modes and effects of action of model brominated flame retardants (BDEs, HBCD, TBBPA) from organophosphate ones (TPhP, TCEP).

8.04.P-Th306 Accounting for Individual Environmental Conditions in Assessing Pollution Exposure of a Long-Lived Top Predator Population (P)

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Environmental stressors originating from both natural (e.g. food availability) and anthropogenic (e.g. synthetic pollutants) sources may have strong influence over extended periods on long-lived species. Consequently, environmental management needs to account for the cumulative long-term effects of multiple stressors on such species. Additionally, when population sizes are reduced with individuals exposed to multiple stressors, the behavior of individuals becomes relatively more important in determining population-level outcomes, these later being more relevant in terms of conservation actions for such species. We developed an individual based toxicokinetic (IBM-TK) model for American alligators as a case study of a long-lived top predator exposed to organochlorine pesticides. The model simulates internal chemical accumulation over the lifetime of individuals, accounting for dietary uptake, growth dilution and maternal transfer processes. The model furthermore considers the between-individual variability in pollution exposure, growth and reproduction directly stemming from food availability in these highly territorial species. Environmental conditions in lakes occupied by alligator populations (such as air and water temperature as well as food web composition) are also considered. Meanwhile, lifecycle development of alligators was modelled using a dynamic energy budget, calibrated against sparse data on growth during early life stages, nest productivity, and age at reproduction. At last, prior knowledge regarding parameter values were phylogenetically derived from related crocodile species in order to feed the Bayesian inference process leading to the parameter estimates of the whole model. Long-lived top predator populations play an important role in the structure and functioning of ecosystems. With this research we aim to contribute towards quantitative mechanistic understanding of the interactions of such species with environmental pollutants present in their natural environments.

8.04.P-Th307 Chemometers: An Integrative Tool for Studying Chemicals in Biota and Surrounding Abiotic Media (P)

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Chemometers are a common and well-defined polymer reference phase for passive equilibrium sampling of a large range of organic pollutants in different matrices like biota, sediment and water. By bringing the chemometer into direct contact with the sample, the chemicals partition between the sample and the polymer until a thermodynamic equilibrium is established. As the chemometers are defined as a common reference phase, the chemical concentrations in the chemometers at equilibrium can be directly compared between different biotic and abiotic compartments and organisms of different trophic levels. This approach has opened a new analytical window for determining the chemical activity of pollutants, which is one of the main drivers for partitioning, biouptake and toxicity. It allows to express the data on a common basis, as the equilibrium partitioning concentrations in the silicone, circumventing normalization, and the extracts of the chemometers can both be submitted to chemical analysis and/or to bioanalytical profiling. The chemometers have proven to be a relevant tool to evaluate chemical pollutants in biota, and we are currently applying them in diverse applications, amongst others to study (I) the thermodynamics of bioaccumulation in aquatic environments using multimedia sampling in a remote Swedish lake, (II) the internal exposure in marine mammal tissues from the German North and Baltic Sea coasts, and (III) passive sampling in the abiotic compartments sediment and water from German rivers with different patterns and levels of pollution as a proxy for chemical concentrations in biota. This presentation will give an overview of our related activities and applications of chemometers aimed at characterizing the complex environmental exposure by mixtures of pollutants at high trophic levels using a common reference phase. An outlook will cover future plans for applications to describe the lifelong exposure to mixtures of pollutants, the so-called exposome, of top predators.

- A** Aagaard, Alf 4.18.P-Th148
 Abang, Eno Williams 4.1.V-08
 Abayomi, Folawewo 3.12.V-03
 Abbasi, Naem 2.03.P-Mo092
 Abel, Sebastian 3.05.P-Tu114, 4.04.P-Th138
 Abele, Cedric 1.02.P-Th024
 Abreu, Sizenando 1.01.P-Mo031
 Achar, Jerry 1.03.P-We011
 Adams, Elena 1.02.P-Th012
 Adams, Nick 5.05.P-Th173
 Adeniji, Catherine 8.04.V-05
 Adeola, Adedapo 3.12.V-01
 Aderjan, Eva 4.10.P-Tu211, 4.10.T-08
 Adrianto, Lugas Raka 6.01.V-03
 Afolabi, Omobolaji 3.19.V-02
 Akoueson, Fleurine 3.05.T-03
 Al Shuraigji, Asma 4.13.P-Tu264
 Alasonati, Enrica 3.18.P-Tu196
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- N** Nantes Université 2.03.T-09, 2.04.P-Tu064, 3.10.P-We076, 7.01.P-Mo276
- National Agriculture and Food Research Organization 5.03.V-05
- National and Kapodistrian University of Athens 3.05.P-Tu118, 3.17.P-Tu183, 4.17.P-Tu283, 7.02.PC-Tu07, 8.04.P-Th301
- National Distance Education University (UNED) 1.01.T-09, 2.06.P-Th040, 2.06.P-Th048, 2.06.P-Th049
- National Institute for Agricultural and Food Research and Technology (INIA-CSIC) 1.08.P-Tu023, 1.08.P-Tu028
- National Institute for Environmental Studies (NIES) 2.04.V-01, 3.05.V-01, 3.14.V-02, 4.16.V-02
- National Institute for Public Health and the Environment (RIVM) 1.03.P-We004, 3.03.V-01, 5.04.P-We242, 6.05.P-We269
- National Institute of Advanced Industrial Science and Technology (AIST) 5.04.V-01
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- National Institute of Standards and Technology 3.02.P-We057, 3.09.P-Mo155, 3.16.P-Th113, 3.18.T-02
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- Newcastle University 3.19.T-15
- NGI 3.14.P-We116, 3.14.P-We135
- NILU - Norwegian Institute for Air Research 3.04.T-04, 3.08.P-Th066, 3.10.T-09, 3.16.P-Th122, 6.07.P-We282, 6.07.P-We283, 8.04.P-Th280
- NILU - Norwegian Institute for Air Research 3.16.T-03
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- NIVA - Norwegian Institute for Water Research 1.01.P-Mo017, 1.02.P-Th308, 1.03.T-03, 2.01.V-05, 2.05.V-02, 2.07.T-01, 3.01.P-Mo101, 3.10.P-We088, 3.10.T-04, 4.03.P-We173, 4.03.PC-We04, 4.13.T-06, 4.14.P-We225, 4.18.P-Th152, 6.05.P-We275, 8.04.P-Th229
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- North West University 3.09.P-Mo192
- North West University (Potchefstroom Campus) 1.02.P-Th010, 3.19.P-We161
- North-West University 3.19.P-We138, 3.19.P-We145, 3.19.T-11, 4.18.P-Th151
- North-West University - Potchefstroom Campus 1.05.T-01, 1.07.T-05, 2.03.P-Mo091
- North-West University - School of Biological Sciences 3.19.P-We160, 4.03.P-We174
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- Norwegian Inst. for Air Research 3.03.P-Mo121
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- Norwegian Institute for Water Research 1.02.T-09, 3.01.P-Mo098, 3.10.P-We078, 8.04.P-Th268
- Norwegian Institute for Water Research (NIVA) 1.02.P-Th015, 3.09.P-Mo148, 3.09.T-05, 4.03.PC-We03, 4.13.P-Tu237
- Norwegian Institute of Bioeconomy Research (NIBIO) 3.08.P-Th060, 3.08.P-Th061, 3.08.P-Th064
- Norwegian Institute of Water Research 3.09.P-Mo145, 3.09.P-Mo147
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- Norwegian University of Science & Technology 1.08.P-Tu012
- Norwegian University of Science & Technology (NTNU) 1.08.P-Tu011, 1.08.V-05, 5.04.T-02, 7.02.T-05
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- Norwegian University of Science and Technology (NTNU) 2.03.P-Mo083, 5.04.P-We239, 6.06.P-Tu301, 6.06.P-Tu302, 7.02.P-Tu312
- NOVA School of Science and Technology 4.18.V-01
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- O** Oekotoxzentrum Eawag-EPFL 1.07.P-We013, 1.08.V-03, 4.16.P-Mo239
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- Örebro University 2.05.P-We025, 2.08.PC-Tu22, 3.09.P-Mo184, 3.19.P-We150, 6.01.PC-We09, 8.04.P-Th213
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- Radboud University Nijmegen 4.13.T-05, 4.17.T-02, 5.05.P-Th165, 6.05.T-04
- RECETOX, Faculty of Science, Masaryk University 1.05.P-Mo048
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- Research Centre for Toxic Compounds in the Environment (RECETOX), Faculty of Science, Masaryk University, Brno, Czech republic 1.01.T-10
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- Research Scientist, Environment and Climate Change Canada 2.03.T-06
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- Ricerca sul Sistema Energetico - RSE S.p.A. 8.04.P-Th254, 8.04.P-Th277
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- RSMAS, University of Miami 2.06.P-Th051
- Ruđer Bošković Institute 1.08.P-Tu027, 3.19.P-We154, 3.19.T-14, 4.13.P-Tu276
- RWS 3.04.P-Tu104
- RWTH Aachen University 1.02.P-Th020, 3.01.P-Mo117, 3.03.P-Mo133, 3.05.P-Tu117, 3.17.P-Tu185, 4.10.T-03
- RWTH Aachen University, Institute for Environmental Research 1.02.P-Th004, 3.06.P-Tu142
- RWTH University Aachen 1.01.P-Mo009
- S** Safer Chemical Analytics LLC 7.03.V-02
- Saint-Petersburg Scientific Research Center for Ecological Safety Russian Academy of Sciences 1.01.V-04
- San Francisco Estuary Institute 4.16.V-01
- San Jorge University 2.05.P-We033, 4.11.P-We207, 4.11.P-We208, 4.13.P-Tu263
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- Simon Fraser University 1.08.T-05, 3.01.P-Mo095, 3.01.P-Mo108, 3.01.T-06, 3.01.T-09
- Simon Fraser University and ECCO 2.03.P-Mo087
- SINTEF Ocean 3.04.P-Tu077, 3.19.P-We148, 3.19.T-13, 4.01.P-Mo215, 4.01.T-04, 4.12.P-We213, 6.08.V-02
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- STATION DE RECHERCHES Sous-marines et Océanographiques 3.03.P-Mo138
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- STIFTINGA VESTLANDSFORSKING 5.03.V-06
- Stockholm University 3.02.P-We053, 3.02.P-We059, 3.04.P-Tu081, 3.05.P-Tu112, 3.06.T-01, 3.06.T-02, 3.07.T-04, 3.12.P-Th090, 4.14.P-We226, 6.05.P-We273, 8.04.P-Th300
- Stockholm University / Science for Life Laboratory 7.02.PC-Tu08
- Stockholm University ACES 3.14.PC-We19, 3.16.T-05
- Stockholm University, ACES 3.03.T-02, 3.05.T-01, 4.14.T-03, 6.04.T-02
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- Swansea University 8.04.P-We319
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- Swedish University of Agricultural Science 3.05.T-05, 3.12.P-Th094, 3.15.T-01
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- Swiss Centre for Applied Ecotoxicology 1.07.T-02, 4.10.P-Tu233, 4.10.P-Tu234, 4.13.V-01
- Swiss Federal Laboratories for Materials Science and Technology (Empa) 8.04.P-We314
- Syddansk Universitet 1.05.T-05
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- Synthesis Environmental LLC 3.03.V-02
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- T** Tallinn University of Technology, School of Engineering 3.04.T-03
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- Technical University of Cartagena 4.10.P-Tu224, 4.10.P-Tu225, 4.10.T-06
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- University of Coimbra 4.10.T-01
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- University of Coimbra, MARE - Marine and Environmental Sciences Centre, Department of Life Sciences, Cal ada Martim de Freitas, 3000-456 Coimbra, Portugal 1.01.P-Mo030
- University of Connecticut 3.08.P-Th059
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- University of Eastern Finland 3.05.P-Tu115, 3.05.T-06, 4.01.P-Mo207, 4.02.P-Mo218, 4.16.T-08
- University of Exeter 3.09.T-01, 3.1.V-16, 3.10.T-27, 6.07.T-04, 6.08.P-Mo265, 8.04.P-Th303, 8.04.P-We304
- University of Extremadura 3.06.P-Tu143
- University of Franche-Comte 2.03.V-09
- University of Gdansk 2.04.P-Tu058, 2.04.P-Tu059, 8.04.P-Th289
- University of Geneva 2.04.V-03, 3.19.T-07
- University of Ghent 3.18.P-Tu191
- University of Girona 3.11.P-Tu160, 3.19.P-We158
- University of Gothenburg 2.01.T-01, 2.01.T-02, 4.18.P-Th153, 6.05.T-01, 8.04.P-Th230, 8.04.P-Th279
- University of Gothenburg, Sweden 3.10.P-We094
- University of Heidelberg 1.05.P-Mo038, 1.05.P-Mo039, 1.05.P-Mo040, 1.05.P-Mo041
- University of Helsinki 4.13.P-Tu259
- University of Hildesheim 2.01.V-04, 2.03.V-08
- University of Hormozgan 3.09.P-Mo183
- University of Insubria 1.03.T-06, 3.01.P-Mo111
- University of Johannesburg 8.04.P-Th278
- University of Kassel 3.16.P-Th110
- University of Koblenz Landau, iES Landau, DFG Graduiertenkolleg Systemlink 2.07.T-03
- University of Koblenz-Landau 2.05.T-03, 2.07.P-We043, 2.07.T-02, 4.02.P-Mo221
- University of Koblenz-Landau, iES Landau, Institute for Environmental Sciences 3.10.P-We101
- University of Lausanne 1.02.V-06, 1.07.P-We018, 1.07.P-We019, 2.04.P-Tu068, 3.01.V-01, 3.08.PC-We17
- University of Leeds 4.13.P-Tu239, 4.13.P-Tu240, 4.13.T-09
- University of Leicester 3.03.P-Mo132
- University of Liege 3.17.P-Tu186, 5.02.P-Mo259, 5.05.P-Th180
- University of Ljubljana 3.10.P-We103, 4.06.P-We203
- University of Ljubljana, Biotechnical Fac. 3.1.V-08
- University of Ljubljana, Faculty of Health Sciences 3.15.P-Tu167, 3.15.P-Tu178
- University of Lleida 3.10.P-We099
- University of Luxembourg 3.02.T-07, 3.16.T-04, 4.12.T-01
- University of Manitoba 3.05.P-Tu129, 8.04.P-Th232, 8.04.P-We296, 8.04.P-We299, 8.04.P-We312
- University of Melbourne 3.04.T-06
- University of Miami 2.05.P-We031
- University of Michigan 1.02.P-Th032, 4.09.V-01, 8.04.V-03
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- University of Namur 4.13.P-Tu253
- University of Naples Federico II 7.01.P-Mo275
- University of Nebraska Lincoln 3.10.P-We077
- University of Nevada, Reno 1.03.P-We007, 3.03.P-Mo130, 3.14.V-01
- University of Nottingham 2.04.P-Tu070
- University of Novi Sad Faculty of Sciences 1.08.P-Tu022
- University of Oldenburg, ICBM 4.07.PC-Tu02
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- University of Osnabruck 2.02.T-02
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- University of Ottawa 1.02.P-Th007, 4.17.P-Tu279, 4.17.T-04, 8.04.P-Th238, 8.04.V-14
- University of Padova 5.03.P-Tu288, 5.04.P-We240, 5.07.V-01
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- University of Puerto Rico at Bayamon 1.01.P-Mo008
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- University of Santa Cruz do Sul 1.07.P-We015, 4.13.P-Tu274
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- Y** Yongin University 1.02.V-01, 4.07.V-02, 4.18.V-02
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- Z** Zurich University of Applied Sciences 3.09.T-02

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